University of Alberta

Population Genetic Studies of Wood and Plains Bison Populations

by

Gregory Allan Wilson ©

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in

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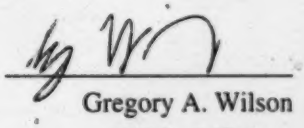

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" 'Where is the herd of buffaloes which was chased by the panther across this plain no later than the morning of yesterday! It is as hard --'

'Friend', said Dr. Battius, who had hitherto been an attentive listener, but who now felt a sudden impulse to mingle in the discourse, 'I am grieved when I find a venator, or hunter, of your experience and observation following the current of vulgar error. The animal you describe is in truth a species of the *Bos ferus* (or *Bos sylvestris*, as he has been happily called by the poets), but though of close affinity, it is altogether distinct from the common bubulus. Bison is the better word, and I would suggest the necessity of adopting it in future when you shall have occasion to allude to the species.'

'Bison or buffalo, it makes but little matter. The creatur' is the same, call it by what name you will, and --'

'Pardon me, venerable venator; as classification is the very soul of the natural sciences, the animal or vegetable must of necessity be characterized by the peculiarities of its species, which is always indicated by the name --'

'Friend,' said the trapper a little positively, 'would the tail of a beaver make a worse dinner for calling it a mink; or could you eat of the wolf with relish because some bookish man had given it the name of venison?' "

James Fenimore Cooper (1827) *The Prairie*

" I remember buffalo
and I remember Hengelo
It would seem to me
I remember every single … thing I know"

The Tragically Hip (1992) *At the Hundredth Meridian*

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Population Genetic Studies of Wood and Plains Bison Populations* submitted by Gregory Allan Wilson in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Systematics and Evolution.

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Abstract

DNA microsatellites are useful tools for examining ecological questions at the individual and population level. I developed a system using a suite of microsatellites in bison (*Bison bison*) to address a number of these issues, specifically: the amount of diversity present in various North American bison populations, the relationships among these populations, and the factors that affect reproductive success in male and female bison.

In spite of the large population bottleneck undergone by bison in North America about 150 years ago, levels of variation are not unlike those of other North American ungulates. The exception to this is Antelope Island State Park, Utah, which has significantly less variation than other bison populations. The population at Custer State Park, South Dakota, was the most variable population. However, diversity in Custer State Park may have been inflated by the hybridization of this population with cattle. When relationships among bison populations were examined, assignment tests and genetic distances showed that wood bison (*Bison bison athabascae*) populations were all more similar to one another than they were to any plains bison population. The Wood Buffalo National Park wood bison population is infected with tuberculosis and brucellosis. A goal of wood bison conservation in Canada is the establishment of wood bison herds free of these diseases, and the latest attempt at this is the Hook Lake Wood Bison Recovery Project, established in 1996. The genetic diversity in this population was

found to be greater than all other wood bison populations, except for Wood Buffalo National Park. Parentage tests performed on the calves born into the Hook Lake Wood Bison Recovery Project population showed that, so far, most of the breeding has been done by only a few males. If a number of males continue to be unsuccessful, the genetic diversity they contain will be lost. Parentage tests were also performed on 317 wood bison calves from Elk Island National Park. Age, weight, prior reproductive success and environmental differences from year to year were found to affect female reproductive success. Only weight and prior reproductive success affected male reproductive success, and left much of the variation in success unexplained.

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First and foremost, I would like to thank Curtis Strobeck for his supervision and helpful comments. Without his frequent support, this thesis would not be what it is.

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Chapter 1

General Introduction

The recent realization that populations can often be characterized by differences in their genetic makeup, and that genetic variation can have a great effect on the health of a population, has resulted in the field of conservation genetics. Conservation genetics is quickly evolving, mainly due to the increase in available technologies used in genetic studies. These technological advances have increased the ability of genetics to address conservation issues.

There are a number of conservation issues that genetic studies can address. One such issue is the phylogeny of various organismal groups. Genetic techniques can be used to identify groups that warrant special conservation efforts as a result of their unique phylogenetic status (see for e.g. Karl and Bowen 1999). Conservation genetics can also be used as a management tool at the level of wild populations or metapopulations by identifying genetic differences between populations. Genetics can be used to determine the relationships between populations, identify the amount of gene flow between them, and investigate historical population reductions. Conservation genetics can also be beneficial for captive herd management by allowing evaluation of the loss of genetic variation through time, and identifying breeding pairs within a population. However, the ability of genetics to examine these questions depends on the tool being used.

Allozyme studies are a common tool in determining differences between populations. While these have been effective in examining the genetic variation in some populations, their usefulness is constrained in that they are not neutral markers, and make up only 5-10% of the nuclear genome. Mitochondrial DNA (mtDNA) is more variable

than most nuclear markers, and is useful in examining phylogenetic questions. However, these studies too are constrained in scope, as the mitochondria acts as a single locus, and its maternal inheritance does not allow for an examination of male mediated gene flow. Highly variable nuclear markers such as microsatellites and minisatellites are also commonly used in conservation genetics. They allow for individual identification and studies of parentage, as well as the examination of population-level questions such as identifying levels of gene flow. An example of their usefulness can be found in a study of northern elephant seals (*Mirunga angustirostis*) by Lehman et al. (1993). This species increased in size from about 100 individuals in the late 1800s to about 125 000 by 1989. Likely as a result of this bottleneck, no genetic variation was found in a study of allozymes in this species. However, minisatellites were able to detect low levels of variation in the northern elephant seals. The history of bison (*Bison bison*) suggest that microsatellites may also be useful in examining conservation genetic issues in this species.

Differentiation of Wood and Plains Bison

The species *Bison* is split into two different subspecies: plains bison (*Bison bison bison*), and wood bison (*Bison bison athabascae* Rhoads 1898). Wood bison are the northern variant of bison in North America, and once existed in northern Alberta, Northwest Territories, Yukon, and Alaska (Van Zyll de Jong 1986, Guthrie 1990, Stephenson et al. 2001). Some behavioural differences have been described between wood and plains bison. Wood bison form smaller groups than plains bison (Soper 1941, Melton et al. 1989), tend to use more forested habitats, and are considered more skittish (Roe 1970).

However, the original definition of wood bison as a separate subspecies is based on morphological characters. Van Zyll de Jong (1986) outlined a number of morphological characters that can be used to differentiate wood and plains bison. Wood bison have an angular hump that has its highest point well in front of the shoulder, while the plains bison's hump is more gently sloping, and peaks at or behind the shoulder. Plains bison have a sharply demarcated cape that is lighter in colour than the rest of the body. The demarcation between cape and torso is less distinct in wood bison, and the cape and body are uniform in colour. Plains bison have distinct chaps on their front legs. In contrast, the hair on a wood bison's front legs is short. Plains bison also have a long, blunt beard, while wood bison have a shorter, pointed beard. Wood bison are about 1/3 larger than plains bison (Olson 2001). Photographs of wood and plains bison can be found in Figure 1-1.

Bison Recent History

For a time, bison were synonymous with life on the plains of North America. Pre-settlement bison numbers have been estimated at 30 to 60 million, although none of these calculations stand up to close scrutiny (Seton 1929, McHugh 1972, Shaw 1995). What is certain, is that by the 1870s, bison numbers had been decimated to a low of about 1000 animals scattered throughout the western plains (Roe 1970). However, with the help of an intense restoration program by the governments of Canada and the United States, numbers of bison in public herds have increased to about 20 000 (Public Herds Census 1993). This bottleneck has had an unknown effect on the genetic variation in this species.

At the time of the near-extirpation of bison, only two pockets of naturally occurring herds remained. Between 22 and 50 plains bison existed in Yellowstone National Park, Wyoming, (Meagher 1973) and about 250 wood bison could be found in what is now Wood Buffalo National Park, Northwest Territories (Soper 1941). Most of the remaining animals were in the hands of ranchers, who had captured small numbers of bison from various regions and used them to start their own herds. Exchange of bison between these ranchers occurred frequently, as they tried to increase the diversity in their herds. All of the other current public bison herds were founded from animals originating from these bison ranchers, and animals were also added to the Yellowstone National Park population. However, the movements of animals between herds in the last 130 or so years have clouded the relationships between some of today's bison populations. Bison movements between ranchers and public herds are summarized in Chapter 2 of this thesis.

The history of wood bison conservation is discussed in Chapter 3. Efforts to protect wood bison were initiated in 1877, with the passing of the Buffalo Protection Act (Hewitt 1921). Another major step in wood bison conservation was the establishment of Wood Buffalo National Park in 1922 to protect the last remaining wood bison (Soper 1941). Unfortunately, a number of plains bison, infected with tuberculosis and brucellosis, were shipped up to this park between 1925 and 1928 (Ogilvie 1979). These bison hybridized before any animals could be removed from Wood Buffalo National Park to found other populations. However, the effect of the introgression of plains bison genes into the wood bison population on the relationships between these groups remains unclear (Geist 1991, Van Zyll de Jong et al. 1995). These aspects of the recent history of bison populations in North America have led to a number of conservation questions about their

levels of variation and relationships which could be answered using molecular techniques.

Previous Studies of Bison Genetics

Prior to the initiation of my research in 1995, there had been a few studies of bison using different genetic systems. Most of these examined the phylogenetic differences between wood bison, plains bison, and cattle. Some of the initial studies used blood groups and other proteins (Stormont et al. 1960, Naik and Anderson 1970, Peden and Kraay 1979). Karyotype studies were also performed in an attempt to answer the same questions (Bhambhani and Kuspira 1969, Ying and Peden 1977). On the whole, the absence of variation in these systems could not resolve the taxonomy of these groups. Later research examined restriction fragment length polymorphisms (RFLPs) of various nuclear genes (Bork et al. 1991, Cronin and Cockett 1993, Morris et al. 1994). Again, few differences were found with this method to establish taxonomic differentiation. Surveys of various bison populations performed using blood groups or mtDNA also found little genetic variation (McClenaghan et al. 1990, Polziehn et al. 1996). Therefore, I pursued genetic studies of bison using a more variable system - DNA microsatellites - in order to increase the ability of molecular techniques to address bison conservation genetic questions.

DNA Microsatellites

DNA microsatellites are members of the variable number of tandem repeat (VNTR) class of loci. This class also includes minisatellites. They are made up of a series of nucleotides (generally one to six) repeated in tandem, occurring at various regions throughout the genome. Microsatellites vary in the number of copies of these repeats. Attention was first called to these markers in 1989 (Litt and Luty 1989, Tautz 1989, Weber and May 1989). While microsatellites were first used in linkage studies (see for e.g. Weissenbach et al. 1992), they have since been used in forensics and individual identification, parentage studies, and population genetics.

Microsatellites are a valuable tool in these studies for a number of reasons. They are generally thought to be selectively neutral (Jarne and Lagoda 1996). The structure and neutrality of microsatellites allow them to be highly variable, even in species where genetic variation is limited or absent in other systems (Hughes and Queller 1993). They are short enough that they can be amplified using the polymerase chain reaction (PCR), so that lower quality and quantity DNA can be used. PCR primers can be designed to amplify only a single microsatellite locus, and in this way the alleles an individual possesses at each locus can be easily determined.

Project Introduction

The variability of microsatellite loci allowed me to address a number of issues facing bison in North America. Chapter 2 examines the amount of genetic variation present in a number of North American bison populations, and determines the relationships among

these populations. Bottlenecks are known to decrease the amount of genetic variation in a species (Wright 1931, Nei et al. 1975). The large population bottleneck undergone by bison 130 years ago has raised concerns about the amount of genetic variation present in this species. Loss of genetic variation has been putatively linked to inbreeding effects (O'Brien et al. 1985). However, many populations exist where low genetic variability does not seem to negatively affect fitness (see for e.g. Paetkau and Strobeck 1994, Houlden et al. 1996). The varied history of North American bison populations should result in different levels of relatedness among them, as populations with similar backgrounds should be more closely related to one another than others. If population sizes and time since separation are equal, heterogeneity should be greater between populations which have little gene flow than between those that have extensive animal exchange (Hartl and Clark 1989). Of special interest was the relationship between different wood and plains bison populations.

Chapter 3 examines the relationship between a wood bison population founded from 1996-1998 at the Hook Lake Wood Bison Recovery Project, and the other wood bison populations. Wood bison are currently considered threatened in Canada (COSEWIC 1998) and exist in large numbers in only three populations: Mackenzie Bison Sanctuary (Northwest Territories), Wood Buffalo National Park (Northwest Territories and Alberta), and Elk Island National Park (Alberta). Unfortunately, as previously mentioned, bison in Wood Buffalo National Park are infected with tuberculosis and brucellosis, and the population has been declining in size. A Federal Environmental Assessment Panel has recommended that Wood Buffalo National Park be depopulated and replaced with healthy animals from other sources (1990). One of the roles of molecular genetics should be the characterization of genetic diversity in populations, so that potential loss of diversity can be monitored through time (Avise 1989, O'Brien

1994). As such, genetic diversity present in Wood Buffalo National Park should be characterized and represented in other populations so that it is not lost forever. To this end, I examined the genetic diversity present in the Hook Lake Wood Bison Recovery Project and other wood bison populations to determine if the genetic diversity present in Wood Buffalo National Park is represented in the other populations. I also examined the paternity of calves born into the Hook Lake Wood Bison Recovery Project, to determine whether this population may be susceptible to inbreeding effects and loss of genetic diversity over time.

Since the first published studies of wild populations in 1992, it has become clear that microsatellites are useful in detecting paternity and hence, reproductive success of males (Ellegren 1992). While studies had been performed on mating success in bison (see for e.g. Lott 1979, Komers et al. 1992) to date no studies of reproductive success have been performed. Reproductive success is a better measure of the ability to produce offspring than mating success, as mating does not guarantee an individual's genes will be passed on to the next generation. Male fertility may vary. Bison can mate at any time of the day, making visual identification of all mating pairs difficult (Lott 1979). Also, some bison cows mate more than once when receptive (Lott 1981), and mating is not necessarily indicative of estrous (Komers et al. 1994). Parentage can then be difficult to ascertain for some individuals. Population genetics techniques can establish parentage without visual identification of mating pairs. Most studies of bison mating behaviour have examined plains bison populations. Since the reproductive success of the individuals in a population will affect the amount of genetic variation lost through time via genetic drift, establishing reproductive success in the threatened wood bison is also important. In Chapter 4, parentage in the wood bison population at Elk Island National Park over a four-year study period was established. This allowed information to be

collected on the number of individuals contributing to the gene pool each year, the age of animals that are reproducing, and the effect various factors have on an individual's ability to reproduce. This information would be useful in determining management strategies for the conservation of this subspecies, especially in captive populations where breeding programs may have to be initiated.

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Figure 1-1A: Plains bison at Elk Island National Park

Figure 1-1B: Wood bison at Elk Island National Park

Chapter 2

Genetic Variation Within and Relatedness Among Wood and Plains Bison Populations[1]

Introduction

Before the European settlement of North America, bison were among the most abundant local fauna. Populations of hundreds of thousands of animals roamed throughout most of North America. The amount of animal exchange between these herds is unknown, but has been suggested to be extensive (Roe 1970). Wood bison (*Bison bison athabascae*) existed in British Columbia, Alberta, the Northwest Territories, Yukon, and Alaska and plains bison (*Bison bison bison*) inhabited most of the remaining prairie regions. The overlap of their ranges, if any, is unknown. Bison numbers were reduced to approximately 1000 by the late 1800s (Roe 1970), about 300 of which were wood bison in what is now Wood Buffalo National Park (Alberta and Northwest Territories). The only other population containing indigenous animals is Yellowstone National Park (Wyoming), where between 22 and 50 animals existed in 1902 (Meagher 1973). The other remaining bison were found in a number of private herds throughout North America. Due to an intense restoration program by the governments of Canada and the United States, over 20 000 bison are currently found in public herds. However, the history of today's bison populations has raised concerns over the amount of genetic variability they contain.

Genetic variation is known to be greatly affected by the founder effect (Wright 1969; Nei et al. 1975). Populations originating from a small number of founders are

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[1] A version of this paper has been published. Wilson, G.A., and Strobeck, C. 1999. Genome 42: 483-496.

expected to contain less genetic variation than those started from a larger number. Most of the public bison populations were founded from few individuals. Bison used to found these herds were mainly from ranch herds started from few animals themselves, further decreasing the amount of genetic variation expected in these herds. In an attempt to decrease the effects of inbreeding (and increase genetic variation), some herds were founded with bison from more than one region (hereafter referred to as different "strains"). A lack of genetic variation has been linked to inbreeding effects in some cheetah populations (O'Brien et al. 1985). However, not all genetically depauperate populations are affected by inbreeding depression (see for e.g. Paetkau and Strobeck 1994), and inbreeding does not seem to decrease fecundity in some bison populations (Berger and Cunningham 1995). The amount of genetic variation present in a number of wood and plains bison populations will be examined.

The history of bison populations also affects the relatedness among them. Populations started with animals from similar locations should be more genetically related than those with different strains. Therefore, one would expect to find larger genetic distances between wood and plains bison, especially if they are different subspecies.

However, the subspecific designation for wood bison is in doubt. Wood Buffalo National Park was created to protect the last remaining wood bison population. Unfortunately, a large herd of plains bison was moved to the Pine Lake region of Wood Buffalo National Park from 1925-1928, and the wood and plains bison in the park hybridized. A herd of what was thought to be pure wood bison was found in a secluded area of Wood Buffalo National Park, and animals were taken from this area to start herds of wood bison in Mackenzie Bison Sanctuary (Northwest Territories) and Elk Island National Park (Alberta) in 1963 and 1965, respectively. Since that time, morphological and genetic evidence has shown that the bison used to start these herds had hybridized, at

least to some extent, with the plains bison (Van Zyll de Jong 1986; Polziehn et al. 1996). Geist (1991) has argued that this hybridization has led to the extinction of wood bison while Van Zyll de Jong et al. (1995) believe that wood bison are still different enough from plains bison to warrant subspecific status. If *Bison bison athabascae* does in fact exist, we would expect to find these populations more genetically differentiated from *Bison bison bison* than from each other.

There may be regions of Wood Buffalo National Park which contain bison that are close to pure wood bison, and other regions which are mostly plains bison. Van Camp (1989) stated that wood bison may still exist in isolated areas. Van Zyll de Jong et al. (1995) described the bison of the Sweetgrass region of Wood Buffalo National Park as the most morphologically similar to pure wood bison, and Pine Lake individuals as intermediate between wood and plains bison. For this to be true, the bison population at Wood Buffalo National Park would need to be fairly heterogeneous, with little gene flow between regions of the park. The heterogeneity of the Wood Buffalo National Park bison subpopulations can be measured to see if it is possible that pockets of mostly pure wood bison still exist. If the Wood Buffalo National Park population is homogeneous, no pure wood bison could exist in the park.

It has been proposed that the bison indigenous to Yellowstone National Park were actually a type of bison called mountain bison, referred to as *Bison bison athabascae* (Meagher 1973). Again, this taxonomic issue is in doubt (for review, see Roe 1970). Plains bison were also added to the indigenous herd at Yellowstone, which diluted the amount of local input to the gene pool to about 40% (Meagher 1973). If mountain bison did exist in this park, the current population should be genetically distinct from other bison populations which do not contain any mountain bison input in their gene pool, or more similar to wood bison as mountain bison and wood bison share the same subspecific designation.

Highly variable regions of the genome must be used to examine genetic variation, diversity and heterogeneity. Bison contain little to no variation at the chromosomal and protein level (Ying and Peden 1977; Bork et al. 1991; Cronin and Cockett 1993; Stormont 1993). More polymorphism was detected by restriction digesting the control region of the mitochondrial DNA, but still some populations were monomorphic (Polziehn et al. 1996). DNA microsatellites are highly polymorphic nuclear markers (Tautz 1989; Weber and May 1989) and have been used to analyze the genetic relationships among populations (for review see Bruford and Wayne 1993), including those that are genetically depauperate (Hughes and Queller 1993; Paetkau et al. 1995). In this study the genetic variability, diversity and heterogeneity in a number of public North American bison herds were investigated with 11 microsatellite loci.

Materials and Methods

Laboratory Methods

The populations used in this study were: plains bison from Antelope Island State Park (Utah, AISP), Custer State Park (South Dakota, CSP), Elk Island National Park (Alberta, EINPP), Fort Niobrara National Wildlife Refuge (Nebraska, FNWR), National Bison Range (Montana, NBR), Wichita Mountains Wildlife Refuge (Oklahoma, WMWR) and Yellowstone National Park (Wyoming, YNP); wood bison from Elk Island National Park (EINPW), Mackenzie Bison Sanctuary (Northwest Territories, MBS) and Wood Buffalo National Park (Alberta and Northwest Territories, WBNP) and a feral herd of plains bison from Pink Mountain (British Columbia, PM). Table 2-1 summarizes the origins of these herds. EINPP had 45 founders as all other bison were shipped from that park to

Buffalo National Park in 1909. While 18 and 24 animals were shipped from WBNP to MBS and EINPW, respectively, only 16 survived the trip to MBS. All of the adults were destroyed in EINPW to eradicate brucellosis, leaving 11 animals as founders (C. Gates, pers. com.). To test the heterogeneity of the Wood Buffalo National Park population, samples from that park were split into the subpopulations of Garden River (GR), Little Buffalo (LB), Needle Lake (NL), Pine Lake (PL), and Sweetgrass (SW).

Sample sizes from the populations were: 30 from AISP, 32 from CSP, 30 from EINPP, 30 from FNWR, 30 from NBR, 21 from WMWR, 33 from YNP, 36 from EINPW, 28 from MBS, and 81 from WBNP. Of the WBNP samples, 8 were from GR, 13 from LB, 14 from NL, 24 from PL, and 22 from SW. DNA samples from AISP were kindly supplied by Julie Schneider. Tissue samples from PM, and DNA from all other populations were obtained from the DNA repository maintained by the Canadian Parks Service at the University of Alberta. As bison groups are quite fluid, and associations between individuals random, it can be assumed that these are random samples from the populations (Lott and Minta 1983; Van Vuren 1983). DNA was extracted from the PM tissue samples using a QIAamp® Tissue Extraction Kit.

The microsatellite loci used in this study were: BM143, BM1225, BM2830, BM4513 and BMC1222 from Bishop et al. (1994), BOVFSH from Moore et al. (1992), Eth121 from Steffen et al. (1993) and RT9, RT24, and RT27 from Wilson et al. (1997). RT29, also used in the study, is RT1 from Wilson et al. (1997) modified so that the primer sequences are GCCTTCTTTCATCCAACAAA and CCCATCTTCCCATCCTCTT. A FAM, HEX, or TET fluorescent dye group was added to the 5' end of one primer from each of these loci. Where possible, these loci were multiplexed during PCR. Multiplexing in this context refers to the amplification of more than one locus in a single PCR reaction. All PCR reactions contained 2.5 mM $MgCl_2$, PCR buffer (10 mM Tris buffer, pH 8.8, 0.1% Triton X100, 50 mM KCl, 0.16 mg/mL

BSA). and approximately 60 ng DNA. The remainder of the PCR reaction contents, and multiplexes, are given in Table 2-2. The PCR was done on an ABI 9600 thermal cycler. Cycling conditions for the PCR reactions were as follows: 1 min. at 94°C, then three cycles of 30s at 94°C, 20s at 54°C and 5s at 72°C, then 33 cycles of 15s at 94°C, 20s at 54°C, 1s at 72°C, then 30s at 72°C. The PCR amplifications were then visualized with an ABI 373A DNA Sequencer and Genescan 672 software.

Data Analysis

To examine variability in the bison populations, we first determined the allele frequencies of each locus for the populations. Allele frequencies are the prevalence of each type of allele in a population. We then used this information to calculate mean number of alleles, average heterozygosity and overall probability of identity (pI). Mean number of alleles is the average number of alleles a population has present at any given locus. Average heterozygosity is the expected number of individuals having copies of different alleles at any locus. Unbiased expected heterozygosity was calculated at each locus using the formula from Nei and Roychoudhury (1974). This was then averaged over all loci to obtain the mean heterozygosity for each population. Probability of identity is the probability that an individual's genotype will be identical to another individual's in the population, and can be calculated with formula (1)

$$(1) \qquad \sum_i p_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, respectively. We obtained unbiased probability of identity values using a formula from Paetkau et al. (1998). The

overall pI for a population was calculated by multiplying together the probabilities of identity for all surveyed loci in that population. Three methods of measuring the original size of the bison populations were compared to these three measures of genetic variation using Kendall's rank correlation test (Sokal and Rohlf 1995). The three measures of original population size were defined as: i) park founders - the number of animals originally used to found each of the public herds, ii) number in original stock - this value is similar to park founders but cannot exceed the lowest number of founders from the ranch populations, and iii) number of strains - the number of different origins for the bison used to found the park herds.

The Monte Carlo approximation of Fisher's exact test was used to detect deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992). Loci displaying an excess of homozygotes may contain null alleles, known to be present in a number of loci (Callen et al. 1993; Koorey et al. 1993; Paetkau and Strobeck 1995). Allele distributions were compared between populations using a G-test for heterogeneity (Sokal and Rohlf 1995). The G-test was chosen as it makes no assumptions about the method of mutation through which microsatellite alleles are derived. Pairwise comparisons between all populations at all loci were performed, and summed over all loci. The G-test was also used to examine the heterogeneity of the WBNP subpopulations. The assignment test, which compares an individual's genotype to the allele frequencies in all populations and assigns it to the population most likely to contain the genotype, was also calculated (Paetkau et al. 1995).

Statistical measures based on the infinite allele model (IAM), as opposed to the stepwise mutation model (SMM), were stressed as they give more reliable results with microsatellite data (Takezaki and Nei 1996; Paetkau et al. 1997). To obtain the genetic relatedness of the bison populations, Nei's standard (D_S, Nei 1972), Nei's minimum (D_M, Nei 1973), delta-mu squared ($(\delta\mu)^2$, Goldstein et al. 1995) and the genotype likelihood

ratio (D_{LR}, Paetkau et al. 1997) genetic distance methods were calculated between all population pairs, and the subpopulations at WBNP. These measures were chosen because D_S and D_M are popular IAM methods for computing genetic distance. $(\delta\mu)^2$, based on the SMM, was designed specifically for microsatellites. D_{LR} is based on the assignment test. It is the log likelihood of a genotype occurring in a population other than its parent population. For example, a D_{LR} value of two means that genotypes are two orders of magnitude more likely to occur in the parent population than the other population being compared. Programs to calculate all of these genetic distances were designed by John Brzustowski and are available at http://www.biology.ualberta.ca/jbrzusto/GeneDist.html. Unrooted trees from the population genetic distance data were created by PHYLIP 3.572 (Felsenstein 1995), using the neighbour-joining (Saitou and Nei 1987) and Fitch and Margoliash (1967) methods.

Results

Allele frequencies for all of the sampled populations at all loci, and their corresponding heterozygosities and probabilities of identity are given in Table 2-3. A range of variation was seen in the sampled loci. Average number of alleles was highest at locus BOVFSH and lowest at BM4513 with values of 9.18 and 1.36, respectively. Locus RT29 had the highest average heterozygosity with 0.767 and BM4513 the lowest with 0.078. The lowest pI occurred for locus RT29, with a value of 0.092, and highest at BM4513, with a value of 0.869. Some alleles were fixed in various bison populations. Allele 133 at locus BM4513 was fixed in populations EINPP, NBR, PM, WMWR, EINPW, MBS, and WBNP. Allele 275 at locus BMC1222 was fixed in AISP. Alleles unique to a population were also discovered. At locus BOVFSH, allele 312 was found only in the WBNP

population, and allele 325 was present only in FNWR individuals. YNP had two unique alleles, 209 and 211, at locus RT24.

As each locus for each population must be checked for Hardy-Weinberg equilibrium, there were a total of 121 genotype distributions to be examined using the Monte-Carlo approximation of Fisher's exact test. However, eight of the allele distributions were monomorphic, so only 113 tests of the Hardy-Weinberg distributions were calculated. Eight of the genotype distributions were outside of the Hardy-Weinberg expectations at the 10% level and 5 at the 5% level. This number of values out of Hardy-Weinberg equilibrium is expected considering the number of tests performed. When the Dunn-Sidák experimentwise error rate was used (Sokal and Rohlf 1995), two genotype distributions deviated from Hardy-Weinberg equilibrium at the 5% level, one of which also deviated at the 1% level. This was the Eth121 allele frequency for the AISP population. A chi-squared goodness of fit test showed the observed number of heterozygotes for this genotype distribution did not deviate from the expected value at the 5% level. The deviation from Monte-Carlo expectations seems to be due to an inordinate number of 188/200 heterozygotes.

Table 2-4 shows the mean number of alleles, average heterozygosities and overall pI for all populations. The AISP population was the least variable with all three measures. The WBNP population was most variable when examining the mean number of alleles, while CSP displayed the most variation with the other two measures. All three methods of measuring variation showed that MBS and EINPW were both notably less variable than their founder population at WBNP. PM was also less variable than its founder population, EINPP, using all three methods of measuring variation.

The rankings used in the correlation analyses are given in Table 2-5. Rankings were calculated using the values from Table 2-1, but a few assumptions were made: i) as CSP had 800 animals added to it from Wind Cave National Park, the number of founders

for this park were added to the CSP population, ii) for the rankings of the number of animals in the original stock, if the animals were moved more than once before reaching their final destination of the public herd, the smallest number of founders from the moves was considered. For example, in 1914, CSP was founded from 36 animals from Philip, who in turn had started his herd from about 70 Dupree animals. As Dupree had started his herd from seven animals, the number of original animals starting the CSP population was then considered to be seven (excluding those from Wind Cave National Park), iii) the number of strains in a herd was calculated by counting the number of strains in each addition of bison. For example, Mackenzie Bison Sanctuary, founded solely from animals from Wood Buffalo National Park, was assigned the same number of strains as the latter park. The number of strains could not exceed the number of bison added, iv) if the number of animals added to a population was less than the number of founders for the emigrant population, the number of founders was added to the immigrant population instead of the actual number of individuals, v) additions of less than eight animals to a public herd more than twenty years after it was originally founded were ignored, vi) it was assumed that PM could not have a larger founding size than EINPP, as it was started solely from the latter population, so they were assigned a tie in the rankings, vii) it was assumed that YNP had 50 native bison in the park in 1902 (Meagher 1973), and viii) WMWR is known to have been founded from six strains of bison from the New York Zoological Gardens (Coder 1975). As Wind Cave National Park was also founded from New York Zoological Gardens, it was also assumed to have six strains of bison. The number of founders correlated with the mean number of alleles ($p<0.01$) and the overall probability of identity ($p<0.1$), while the number in original stock correlated with the mean number of alleles ($p<0.01$). None of the variability measures correlated with the number of strains per herd.

All pairs of populations had significantly different allele distributions when using the G-test ($p<0.001$). However, of the WBNP subpopulations, only the allele distributions for the NL-GR, PL-GR, PL-LB, PL-NL, PL-SW and NL-SW comparisons differed significantly at the 10% level using the G-test. Only NL-PL and NL-SW were significantly different when $p<0.001$.

Of the 370 individuals used in the assignment test, 276 of them (75%) were assigned to the correct population (Table 2-6). Only five (1.4%) of the incorrectly assigned animals were placed in the incorrect subspecies, and all of these were WBNP bison assigned to various plains bison populations. Thirty-three percent of the WBNP animals were assigned to either EINPW or MBS. Thirty-three percent of the individuals from EINPP were specifically assigned to PM and 32% of the PM bison were assigned to EINPP. FNWR, AISP and WMWR each had all of their individuals assigned to the correct population. Table 2-7 shows the number of individuals from each of the WBNP subpopulations that were assigned to the various populations. Of the five WBNP animals assigned to plains bison populations, three were from SW, one was from PL and one was from LB.

The genetic distances between all population pairs, and the WBNP subpopulations, can be found in Table 2-8. For D_S, the smallest distances between populations were the EINPP-PM and the WBNP-EINPW distances. The largest between population distance was between EINPW and FNWR. The smallest interpopulation distance with the D_M measure was between both WBNP-EINPW and EINPP-PM. The largest D_M distances were EINPW-AISP, and AISP-PM. The D_{LR} genetic distance measure had the smallest interpopulation distance when comparing the EINPP and PM populations and the largest when measuring the EINPW-FNWR distance. $(\delta\mu)^2$ had the smallest interpopulation values when measuring the WBNP-EINPW distance and the largest when measuring the EINPW-AISP and NBR-AISP distances.

Unrooted trees for the populations were designed for all four of these genetic distance measures by PHYLIP 3.572, using the neighbour-joining (Saitou and Nei 1987) and Fitch and Margoliash (1967) methods. The unrooted tree made by applying the Fitch and Margoliash algorithm to the D_M distances is shown in Figure 2-1. Aside from minor differences in branch lengths, both the neighbour-joining and Fitch and Margoliash algorithms gave trees identical to this one for the D_S, D_M, and D_{LR} distance measures, except the D_{LR} neighbour-joining tree has the (FNWR-CSP-NBR) and (WMWR-YNP) branches exchanged. Wood bison form one group on this tree. The unrooted tree created using $(\delta\mu)^2$ was not analyzed, as it was quite different from the other three. The $(\delta\mu)^2$ measure has been found to have high variance, and this could be the reason its results differ from the other three (Paetkau et al. 1997). Takezaki and Nei (1996) also found that the $(\delta\mu)^2$ measure was less reliable than other methods of determining distances using microsatellites.

Discussion

The bison populations exhibited levels of microsatellite variability similar to other mammalian species, especially those recently undergoing population bottlenecks (Roy et al. 1994; Paetkau and Strobeck 1995; Houlden et al. 1996). Bison were also found to have variability levels similar to other large mammalian species in a study of allozymes (McClenaghan et al. 1990). The pre-bottleneck microsatellite variation must surely have been larger than it is today, as bottlenecks lower the amount of genetic variation in a population.

Wood Buffalo National Park was the most variable population when using mean number of alleles, while Custer State Park was the most variable with the other measures.

This may be due to the fact that mean number of alleles tends to increase with number of individuals sampled. Wood Buffalo National Park had over two times the number of sampled individuals than the other populations, which may have increased the number of alleles detected. It is also of interest to note that each of the three sampled populations (Pink Mountain, and Mackenzie Bison Sanctuary and the wood bison at Elk Island National Park) which were started from one of the other sampled populations (plains bison at Elk Island National Park, and Wood Buffalo National Park, respectively) contain less genetic variation than their founding herds. Pink Mountain had a larger number of founders than either of the other two populations, and is closer to its founding population in variability because of this.

The correlations of the mean number of alleles and probability of identity with the number of park founders, and mean number of alleles with the number in the original stock suggest that the amount of variation present in populations is indeed affected by the amount of potentially different genetic material in the populations. The number of founders has more of an effect on the amount of genetic variability in a population than the number of strains in that population does. Therefore, increasing the number of founders for a population is more effective in raising the amount of genetic variability in that population than increasing the number of strains. The failure of mean heterozygosity to correlate with any of the measures of original population size could be due to the fact that a number of the mean heterozygosity values are quite similar and may not be significantly different from one another, resulting in an incorrect ranking.

None of the variability measures were found to correlate to the number of mitochondrial alleles found in a study by Polziehn et al. (1996). It must be remembered that the mitochondria is only a single locus inherited maternally, and as such may not be a good indicator of the amount of variation present in populations which have undergone recent size changes.

Some individuals in the Custer State Park population have cow mitochondrial DNA (Polziehn et al. 1995). Known sizes of the loci used in this study in cattle are given in Table 2-3. Data from Bishop et al. (1994) involve the use of about 200 animals, while Moore et al. (1992) used 19 cattle. Most of the loci, when used in cattle, display a much wider range in allele sizes than bison. None of the bison at Custer State Park contained an allele in their genotype unique to their population, and all the alleles were within the size range found in other bison populations. Therefore, there is no evidence that any microsatellite alleles in the Custer State Park population originated from cattle.

Results of the G-test and the assignment test show that all the sampled bison populations are genetically distinct from one another. The founder effect and genetic drift - resulting from the small number of transfers between herds that have occurred - are probably responsible for the uniqueness of these populations. Most of the incorrect assignations of the assignment test were between Pink Mountain and the plains bison at Elk Island National Park, or between Wood Buffalo National Park and Mackenzie Bison Sanctuary or the wood bison population at Elk Island National Park. These are the only three instances of one sampled herd being directly established from another sampled herd, and all three occurred relatively recently.

The largest distances observed in this study were similar to those between widely separated North American polar bear populations, another mammal with a large home range (Paetkau et al. 1995). Founding effects and limited gene flow inflate genetic distance values, so we would expect genetic distances between bison populations to be larger than those obtained. If the bison inhabiting North America before their near-extinction were essentially acting as a single metapopulation, with gene flow occurring between all areas, genetic distances between areas would be low. We would then expect to see low genetic distances between present herds despite the founding effects that have occurred. The extensive natural exchange of animals between herds on a daily basis

within public parks today (Lott and Minta 1983; Van Vuren 1983) supports the idea of extensive gene flow in the past. Seton (1910) claimed that all of the plains bison present in Canada acted as one herd, at least before 1869. Roe (1970), also impressed with the homogeneity of bison, stated that "in spite of the wide climatic variation, we are confronted with a species which is, broadly speaking, the same throughout this huge territory", with the possible exception of the wood bison. The physical similarity of North American bison should also be reflected in a genetic homogeneity, as is seen here.

As the D_S, D_M and D_{LR} distance measures and both methods of designing trees all resulted in the same unrooted tree, there is some support for the relationships therein. The tree and the D_S, D_M and D_{LR} distance measures all show the plains bison populations at Elk Island National Park and Pink Mountain to be the most closely related. Since the Pink Mountain population was founded recently from a fairly large number of Elk Island National Park individuals, this is not surprising. This suggests that the founding size of 48 animals for the Pink Mountain population was sufficient to obtain a representative sample of the genetic content of the Elk Island National Park population, though the genetic variation at Pink Mountain is smaller. If mountain bison existed and made a significant contribution to the gene pool of the bison at Yellowstone National Park, we would expect this population to be on a branch by itself or amongst the wood bison populations, as both mountain bison and wood bison were considered *Bison bison athabascae*. The genetic distances between the Yellowstone bison and the other populations would also be expected to be larger. As neither of these are supported by our results, the bison indigenous to Yellowstone were probably not mountain bison, but rather plains bison driven to the area by hunters. The relatively large genetic distances between the Antelope Island State Park population and all other bison populations, and its position on a branch by itself on the unrooted tree, could be a result of the extremely low

genetic variability at Antelope Island. Low genetic variation increases the genetic distance between populations, as they may not share the same alleles by chance.

Genetic distances between wood and plains bison populations were larger than those within either of the two proposed subspecies. The three wood bison populations also form one group on the tree, and the genetic distances between these populations are low, relative to other bison populations. This is expected since the Mackenzie Bison Sanctuary and Elk Island National Park wood bison herds were founded solely from Wood Buffalo National Park. This grouping of the wood bison is strong, even after the introduction of numerous plains bison to Wood Buffalo National Park. Wood bison would likely have been even more distinct genetically from the plains bison had the introduction of plains bison to Wood Buffalo National Park not occurred, as the influx of plains bison genetic material to this population would act to "average out" differences between the wood and plains bison populations. The clustering of these three populations implies that wood bison are functioning as entities distinct from plains bison, and should continue to be managed separately. The small genetic distances between these populations supports the idea that the founders of Elk Island National Park and Mackenzie Bison Sanctuary were wood-plains hybrids like the animals at Wood Buffalo National Park, and not pure wood bison.

It may be noted that the wood bison population at Elk Island National Park has larger genetic distances using all measures between itself and plains bison populations, while distances between Wood Buffalo National Park and the plains bison populations are smaller. This does not necessarily mean that the wood bison at Elk Island National Park are most like pure wood bison. This population was essentially founded from 11 individuals, as all of the animals shipped to the park from Wood Buffalo National Park were destroyed and only their offspring were kept. The reduction in founding stock could have increased the genetic distance between Elk Island National Park and all other

populations. The power of the founder effect to lead to genetically different populations is illustrated by the genetic distance between Mackenzie Bison Sanctuary and the wood bison population at Elk Island National Park. These two populations were started at about the same time with animals taken from the same locale, but their genetic distance shows that their gene pools are quite distinct. Distances between Wood Buffalo National Park and the plains bison populations would be expected to be smaller than those of the other wood bison populations as Wood Buffalo National Park has a much higher genetic variability, and would share more alleles with the plains bison populations by chance.

The G-test indicates that the allele frequencies of the Pine Lake subpopulation at Wood Buffalo National Park are significantly different from all other subpopulations, and that the allele distributions at Needle Lake are also different from Sweetgrass and Garden River. All other comparisons were not significantly different. Pine Lake was the area chosen by Van Zyll de Jong et al. (1995) to be the most intermediate between wood bison and plains bison. The Pine Lake region was the site of the initial release of plains bison into the park, and this could have resulted in the uniqueness of this subpopulation, if it contains more genetic input from the introduced plains bison than other regions. However, genetic distances between all the regions of the park are extremely small. This suggests that while there may be differentiation between some of the subpopulations, there is still gene flow between all regions of the park, and no region should be free of genetic input from the introduced plains bison. Of the five animals from Wood Buffalo National Park misassigned with the assignment test to plains bison populations, three were from Sweetgrass, one was from Pine Lake and one was from Little Buffalo. This also suggests that plains bison genetic material occurs throughout Wood Buffalo National Park. Even though Sweetgrass was chosen by external characteristics to be the most like pure wood bison (Van Zyll de Jong et al. 1995), more bison were assigned to plains bison populations from this region than from any other.

The correlation of the founding size of the bison populations with the mean number of alleles and overall probability of identity shows that microsatellites are good tools for examining the recent history of populations. Populations started from small numbers of animals have less genetic variation. Since the number of strains was not correlated with any of the measures of variation, it is more important to use large number of animals irrespective of their origins in order to start herds with high genetic variation. The Pink Mountain population, founded relatively recently from 48 individuals, seems to contain a representative amount of the variation present in its parent population, judging from genetic distance data. This number of founders may then be a minimum level that could be aimed for when new bison populations are started. As the wood bison populations at Elk Island National Park and Mackenzie Bison Sanctuary do not contain as much variation as their founding population, Wood Buffalo National Park, they would not be suitable replacements if the latter population is to be extirpated. The genetic similarity of all wood bison herds existing today, either inside or outside Wood Buffalo National Park, suggests that they all contain some plains bison genetic material in their gene pool.

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Table 2-1. Origins of the bison herds used in this study (Garretson 1938, Rorabacher 1970, Meagher 1973, Dary 1974, Coder 1975, Ogilvie 1979, Jenning and Hebbing 1983, Christainsen 1991, Malcolm 1993, Polziehn 1993, C. Gates, pers. com., R. Walker, pers. com., T. Novak, pers. com.).

Ranch herds			
Owner	**Origin**	**Number**	**Year**
Alloway/McKay	Saskatchewan	5	1873-4
Bedson	Manitoba (?)	3	1880
Bedson	Alloway/McKay	8	1880
Strathcona	Alloway/McKay	all	1887
Goodnight	Texas panhandle	6	1878
Goodnight	a Texas ranch (origin unknown)	1	1878
Goodnight	death, before breeding	-2	1878
Walking Coyote	Montana	4	1879
Pablo/Allard	Walking Coyote	12	1883
Pablo/Allard	Jones	26	1893
Conrad	Pablo/Allard	30	1902
Eaton	Pablo/Allard	60	1902
Dupree	Montana	6-7 (?)	1882
McCoy	Oklahoma (?)	2	1882
PWFC	McCoy	2	1886
Jones	Oklahoma	56	1886-9
Jones	Bedson	all (~75)	1889
Jones	Kansas, Nebraska ranches	10	1889
Corbin	Jones (From Bedson)	12	1889
Corbin	Jones	10	1892
Corbin	Banff NP	2	1904
Whitney	Wyoming (?)	13	1897
Whitney	Jones (from Oklahoma)	1	1897
Philip	Dupree	~75	1901-2
Gilbert	PWFC	3	1902
Gilbert	ranch in Iowa	1	1903

Table 2-1 continued. Origins of the public herds.

Public herds			
Herd	**Origin**	**Number**	**Year**
AISP	Jones (from Bedson?)	12	1893
NYZG	Goodnight	4	1899
NYZG	Oklahoma	3	1899
NYZG	?	3	1900
NYZG	Wyoming	13	1903
NYZG	PWFC	1M, 3F	1904
NYZG	Whitney	13 (?)	1900-04
NYZG	Whitney (Oklahoma Jones animal)	1	1901
Banff NP	Goodnight	3	1897
Banff NP	Strathcona	13	1898
Banff NP	Corbin	2M	1904
YNP	native	22-50	1902
YNP	Eaton	18F	1902
YNP	Goodnight	3M	1902
WMWR	NYZG	6M, 9F	1907
WMWR	FNWR	4M	1942
WMWR	NBR	4M	1952
EINPP	Pablo/Allard	183	1907
EINPP	Banff NP	7M	1907
BNP	EINPP	all but 45	1909
Banff NP	Pablo/Allard	16M	1907
BNP	Pablo/Allard	298	1909-12
BNP	Banff NP	91	1909-14
NBR	Conrad	37	1909
NBR	Goodnight, Corbin, Jones	12	1910-11
NBR	WMWR	4M	1952
NBR	YNP	2M	1953
WCNP	NYZG	7M, 7F	1914
FNWR	Gilbert	6	1913
FNWR	YNP	2M	1913
FNWR	CSP	8	1935-7
FNWR	NBR	5	1952
CSP	Philip	36	1914
CSP	Pine Ridge Reservation (origin unknown)	few	1940s
CSP	WCNP	~800	1950s
MBS	WBNP	16	1963
WBNP	native	200 minimum	
WBNP	BNP	6673	1925-28
EINPW	WBNP	11	1965
PM	EINPP	48	1971

Note: abbreviations: Page Woven Fence Company (PWFC), Antelope Island State Park (AISP), New York Zoological Gardens (NYZG), Yellowstone National Park (YNP), Wichita Mountains Wildlife Refuge (WMWR), Fort Niobrara Wildlife Refuge (FNWR), National Bison Range (NBR), plains bison at Elk Island National Park (EINPP), Buffalo National Park (Wainwright, BNP), Wind Cave National Park (WCNP), Custer State Park (CSP), Mackenzie Bison Sanctuary (MBS), wood bison at Elk Island National Park (EINPW), and Pink Mountain (PM).

Table 2-2. Remainder of the contents and amounts for the PCR reactions. Loci 1A, 1B, and 1C are BM143, BM2830 and BM1225, respectively. RT29, BMC1222 and BM4513 are Primer A, B, and C in reaction mix 2, respectively. RT27, RT24 and RT9 are multiplexed as loci 3A, 3B, and 3C, respectively. Eth121 and BOVFSH are amplified separately using reaction mix 4.

	Reaction Mix 1	Reaction Mix 2	Reaction Mix 3	Reaction Mix 4
Each Primer A	0.19 μM	0.19 μM	0.18 μM	0.16 μM
Each Primer B	0.19 μM	0.19 μM	0.16 μM	-
Each Primer C	0.17 μM	0.13 μM	0.16 μM	-
dNTPs	160 μM	160 μM	160 μM	120 μM
Taq Polymerase	0.68 units	0.48 units	0.6 units	0.6 units

Table 2-3. Allele frequencies, heterozygosities (H) and probabilities of identity (pI) for each population at each locus. The sample size (n) is given in the first table. If known, the size of the loci in cattle (CSize) is also given. Abbreviations can be found in Table 2-1.

Locus BM143 (CSize 90-122)

		Allele Frequencies									
	n	99	101	103	105	109	111	113	115	H	pI
AISP	30	0.017	0.75		0.167	0.033			0.033	0.414	0.37
CSP	32	0.094	0.094	0.109	0.281		0.172	0.031	0.219	0.826	0.053
EINPP	30		0.25	0.1	0.167		0.15		0.333	0.779	0.083
FNWR	30	0.117	0.567	0.083	0.117				0.117	0.642	0.154
NBR	30		0.533		0.2			0.033	0.233	0.631	0.192
PM	19		0.184	0.342	0.132		0.053		0.289	0.765	0.094
WMWR	21		0.286	0.095	0.238		0.381			0.725	0.128
YNP	33		0.212	0.242	0.076		0.333	0.045	0.091	0.781	0.081
EINPW	36	0.014	0.472		0.514					0.52	0.351
MBS	28	0.375	0.339	0.018	0.089	0.071	0.018		0.089	0.736	0.11
WBNP	81	0.179	0.506	0.049	0.167	0.031	0.025		0.043	0.682	0.135

Locus BM2830 (CSize 149-203)

	Allele Frequencies													
	141	143	147	149	151	153	155	157	159	163	165	167	H	pI
AISP			0.25	0.4	0.083				0.067	0.183		0.017	0.745	0.103
CSP			0.156	0.109	0.031	0.234	0.047	0.063	0.297	0.047	0.016		0.824	0.052
EINPP		0.017			0.133	0.083		0.067	0.417	0.25	0.033		0.746	0.098
FNWR			0.117	0.017		0.233		0.033	0.35	0.05	0.2		0.779	0.081
NBR			0.067	0.15	0.017	0.183			0.117	0.4	0.067		0.774	0.077
PM				0.053	0.132	0.184			0.421	0.211			0.744	0.101
WMWR			0.048	0.095	0.238	0.405		0.024		0.19			0.749	0.099
YNP	0.091		0.015	0.045	0.061	0.303			0.212	0.045	0.227		0.807	0.063
EINPW			0.25			0.583	0.139					0.028	0.585	0.228
MBS			0.321			0.5			0.054		0.036	0.089	0.646	0.183
WBNP		0.012	0.216	0.025	0.019	0.481		0.012	0.123	0.037	0.025	0.049	0.705	0.119

Locus BM4513 (CSize 141-161)

	Allele Frequencies			
	133	135	H	pI
AISP	0.917	0.083	0.155	0.72
CSP	0.813	0.188	0.31	0.519
EINPP	1		0	1
FNWR	0.917	0.083	0.155	0.72
NBR	1		0	1
PM	1		0	1
WMWR	1		0	1
YNP	0.864	0.136	0.239	0.601
EINPW	1		0	1
MBS	1		0	1
WBNP	1		0	1

Table 2-3 continued.

Locus BMC1222 (CSize 272-302)

	Allele Frequencies				H	pI
	267	273	275	277		
AISP			1		0	1
CSP	0.094	0.156	0.656	0.094	0.536	0.246
EINPP	0.3		0.7		0.427	0.416
FNWR	0.183	0.05	0.767		0.382	0.418
NBR	0.15	0.033	0.717	0.1	0.46	0.318
PM	0.132		0.868		0.235	0.602
WMWR	0.024	0.024	0.952		0.094	0.816
YNP	0.03		0.97		0.06	0.883
EINPW		0.097	0.444	0.458	0.591	0.258
MBS	0.036	0.054	0.893	0.018	0.202	0.636
WBNP	0.043	0.123	0.525	0.309	0.616	0.212

Locus BM1225 (CSize 227-253)

	Allele Frequencies										H	pI
	238	240	244	246	248	252	264	268	270	272		
AISP		0.717				0.15	0.133				0.454	0.334
CSP	0.141	0.344			0.063	0.234	0.109	0.016	0.094		0.795	0.069
EINPP	0.017	0.467	0.15	0.017	0.083	0.15		0.033	0.083		0.734	0.095
FNWR	0.1	0.633				0.067	0.017	0.167		0.017	0.566	0.217
NBR	0.017	0.383		0.033	0.033	0.117		0.317	0.017	0.083	0.742	0.105
PM		0.368	0.211	0.026	0.053	0.079		0.079	0.184		0.791	0.07
WMWR		0.738				0.167		0.024	0.071		0.432	0.348
YNP		0.5				0.152		0.091	0.258		0.662	0.164
EINPW		0.042			0.111	0.583		0.264			0.584	0.229
MBS		0.196				0.643		0.161			0.532	0.269
WBNP		0.179	0.006	0.093	0.006	0.438	0.006	0.222	0.037	0.012	0.721	0.117

Locus BOVFSH (CSize 291-320)

	Allele Frequencies																	H	pI
	296	298	299	302	303	304	308	309	310	311	312	313	316	317	321	322	325		
AISP	0.017							0.983										0.033	0.933
CSP	0.125	0.125		0.016	0.328	0.047	0.016	0.109				0.125	0.047		0.063			0.838	0.041
EINPP	0.067	0.05		0.05	0.017		0.167	0.2	0.1			0.05			0.25	0.05		0.859	0.035
FNWR	0.167	0.383			0.017					0.167		0.05	0.05		0.1		0.067	0.791	0.066
NBR		0.017		0.683	0.05	0.017	0.017					0.017	0.017		0.183			0.504	0.274
PM		0.105		0.026	0.105		0.211	0.158	0.105			0.053			0.132	0.105		0.889	0.023
WMWR	0.048	0.024		0.095		0.071	0.048					0.429	0.286					0.733	0.106
YNP	0.015	0.152		0.03	0.106	0.182	0.015	0.273		0.136		0.015		0.015	0.03	0.03		0.849	0.039
EINPW			0.056	0.014	0.083			0.528		0.125		0.014		0.042	0.139			0.684	0.122
MBS		0.018	0.036	0.036	0.304		0.018	0.179		0.161		0.036		0.018	0.143	0.054		0.837	0.044
WBNP	0.025	0.025	0.031	0.062	0.123		0.049	0.333	0.012	0.136	0.006	0.043	0.012	0.037	0.093	0.012		0.84	0.039

Table 2-3 continued.

Locus Eth121 (CSize 173-212)

	Allele Frequencies					H	pI
	186	188	194	198	200		
AISP	0.8	0.133		0.017	0.05	0.345	0.447
CSP	0.375	0.453	0.031	0.094	0.047	0.652	0.185
EINPP	0.433	0.1	0.05		0.417	0.637	0.205
FNWR	0.183	0.383	0.283	0.117	0.033	0.737	0.113
NBR	0.55	0.35	0.017		0.083	0.577	0.258
PM	0.237	0.079		0.079	0.605	0.58	0.22
WMWR	0.762	0.071		0.167		0.396	0.393
YNP	0.788	0.03	0.015	0.061	0.106	0.369	0.41
EINPW	0.403			0.111	0.486	0.597	0.249
MBS	0.911				0.089	0.166	0.704
WBNP	0.469	0.049	0.037	0.068	0.377	0.634	0.202

Locus RT9

	Allele Frequencies				H	pI
	113	115	117	119		
AISP	0.95	0.05			0.097	0.817
CSP	0.516	0.141	0.344		0.606	0.233
EINPP	0.617	0.067	0.317		0.524	0.306
FNWR	0.75		0.167	0.083	0.41	0.381
NBR	0.783	0.117	0.083	0.017	0.372	0.412
PM	0.316	0.158	0.526		0.615	0.22
WMWR	0.405	0.143	0.405	0.048	0.666	0.179
YNP	0.394	0.152	0.455		0.625	0.221
EINPW	0.694	0.056	0.25		0.459	0.354
MBS	0.786	0.089	0.125		0.366	0.423
WBNP	0.858	0.043	0.099		0.254	0.571

Locus RT24

	Allele Frequencies								H	pI
	205	209	211	213	225	227	229	231		
AISP	0.967						0.017	0.017	0.066	0.87
CSP	0.656				0.109		0.188	0.047	0.528	0.26
EINPP	0.7			0.05	0.15		0.05	0.05	0.488	0.283
FNWR	0.583				0.383	0.017		0.017	0.521	0.331
NBR	0.5				0.133	0.283		0.083	0.656	0.172
PM	0.868			0.026	0.053		0.053		0.246	0.564
WMWR	0.857				0.071		0.071		0.261	0.549
YNP	0.742	0.015	0.015	0.045	0.03	0.106		0.045	0.439	0.325
EINPW	0.625						0.306	0.069	0.518	0.308
MBS	0.661						0.339		0.456	0.397
WBNP	0.685			0.012	0.025	0.006	0.228	0.043	0.479	0.322

Table 2-3 continued.

Locus RT27

	Allele Frequencies				H	pI
	146	148	150	152		
AISP	0.133		0.867		0.235	0.606
CSP	0.422	0.031	0.453	0.094	0.617	0.227
EINPP	0.033	0.033	0.933		0.129	0.757
FNWR	0.4		0.517	0.083	0.576	0.272
NBR	0.2	0.1	0.65	0.05	0.534	0.257
PM	0.026		0.974		0.053	0.895
WMWR	0.214	0.048	0.738		0.417	0.385
YNP	0.091	0.091	0.818		0.319	0.479
EINPW	0.333		0.667		0.451	0.401
MBS	0.089		0.911		0.166	0.704
WBNP	0.173	0.012	0.802	0.012	0.328	0.488

Locus RT29

	Allele Frequencies								H	pI
	206	212	214	216	218	220	222	224		
AISP	0.233	0.033			0.267	0.433	0.033		0.696	0.147
CSP	0.219	0.234	0.188		0.125	0.156	0.031	0.047	0.832	0.051
EINPP	0.083	0.267	0.117	0.05	0.133	0.133		0.217	0.837	0.047
FNWR	0.117	0.15	0.267		0.417		0.05		0.729	0.115
NBR		0.083	0.067		0.317	0.383	0.033	0.117	0.739	0.108
PM	0.053	0.395	0.053		0.158	0.263		0.079	0.758	0.092
WMWR	0.381				0.024	0.167	0.286	0.143	0.742	0.11
YNP	0.106	0.045	0.03		0.106	0.227	0.197	0.288	0.814	0.061
EINPW	0.25	0.125	0.014			0.167	0.417	0.028	0.73	0.114
MBS	0.036	0.393	0.018		0.018	0.268	0.179	0.089	0.745	0.103
WBNP	0.259	0.198	0.056	0.012	0.056	0.235	0.16	0.025	0.811	0.063

Table 2-4. Mean values for number of alleles, mean heterozygosity, and overall probability of identity (pI), the product of the pI values at each locus, for the bison populations. Abbreviations are given in Table 2-1.

	Mean # Alleles	Heterozygosity	1 / overall pI
AISP	3.18	0.295	4 100
CSP	5.64	0.669	7 600 000 000
EINPP	5.00	0.560	140 000 000
FNWR	4.64	0.572	42 000 000
NBR	4.91	0.544	15 000 000
PM	4.36	0.516	48 000 000
WMWR	3.91	0.474	1 600 000
YNP	5.36	0.542	67 000 000
EINPW	3.64	0.520	1 400 000
MBS	4.27	0.441	760 000
WBNP	6.55	0.552	57 000 000

Table 2-5. Rankings assigned to the sampled bison populations used in Kendall's rank correlation test. The first value is the ranking, and the second number in parenthesis is the actual value for the size of founding population measures. Actual values for the variability measures are in Table 2-4. Abbreviations are given in Table 2-1.

Table 2-5A. Size of founding population measures.

Size of Founding Population Measures					
Park founders		Number of strains		Number in original stock	
FNWR	1 (8)	AISP	1 (2)	FNWR	1 (5)
EINPW	2 (11)	FNWR	2 (4)	AISP	2 (8)
AISP	3 (12)	WMWR	3 (6)	EINPW	3 (11)
WMWR	4 (15)	NBR	4.5 (8)	WMWR	4 (15)
MBS	5 (16)	YNP	4.5 (8)	MBS	5 (16)
EINPP	6.5 (45)	EINPP	7 (8)	CSP	6 (26)
PM	6.5 (45)	PM	7 (8)	PM	7.5 (37)
NBR	8 (49)	CSP	7 (8)	EINPP	7.5 (37)
CSP	9 (55)	WBNP	10 (9)	NBR	9 (42)
YNP	10 (71)	EINPW	10 (9)	YNP	10 (71)
WBNP	11 (240)	MBS	10 (9)	WBNP	11 (240)

Table 2-5B. Variability measures.

Variability Measures					
Mean # alleles		Heterozygosity		Overall probability of identity	
AISP	1	AISP	1	AISP	1
EINPW	2	MBS	2	MBS	2
WMWR	3	WMWR	3	EINPW	3
MBS	4	PM	4	WMWR	4
PM	5	EINPW	5	NBR	5
FNWR	6	YNP	6	FNWR	6
NBR	7	NBR	7	PM	7
EINPP	8	WBNP	8	WBNP	8
YNP	9	EINPP	9	YNP	9
CSP	10	FNWR	10	EINPP	10
WBNP	11	CSP	11	CSP	11

Table 2-6. Assignment test results. Individuals assigned to their source population are in bold. Individuals assigned to the incorrect subspecies are in italics. Abbreviations can be found in Table 2-1.

Source	Population to which the individual was assigned										
	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP
AISP	**30**										
CSP		**30**		1	1						
EINPP	1		**17**	1	1	10					
FNWR				**30**							
NBR	1	1			**28**						
PM			6			**12**		1			
WMWR							**21**				
YNP			1			1	2	**29**			
EINPW									**33**	2	1
MBS										**27**	1
WBNP	*1*	*1*		*1*		*2*			17	10	**49**

Table 2-7. Results of the assignment test for the Wood Buffalo National Park individuals, sorted by subpopulation. The total number of individuals in each subpopulation is in the first column (Total). The number of animals from each subpopulation assigned to WBNP, and all other populations, are in the other columns. Populations not listed had no WBNP animals assigned to them. Abbreviations for the populations can be found in Table 2-1.

					Plains Bison Populations			
	Total	WBNP	EINPW	MBS	AISP	CSP	FNWR	PM
GR	**8**	6	1	1				
LB	**13**	8	2	2		1		
NL	**14**	8	5	1				
PL	**24**	12	7	4				1
SW	**22**	15	2	2	1		1	1

Note: Abbreviations for the subpopulations: Garden River (GR), Little Buffalo (LB), Needle Lake (NL), Pine Lake (PL), Sweetgrass (SW).

Table 2-8. Genetic distances between all bison populations, and the subpopulations at Wood Buffalo National Park (in bold). Distances between Wood Buffalo National Park and its subpopulations were not calculated. Population abbreviations can be found in Table 2-1, and subpopulation abbreviations are in Table 2-7.

Table 2-8A. D_s results are above the diagonal and D_A are below the diagonal.

	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP	GR	LB	NL	PL	SW
AISP		0.261	0.204	0.261	0.215	0.290	0.217	0.184	0.280	0.209	0.171	0.239	0.201	0.221	0.155	0.179
CSP	0.147		0.162	0.151	0.225	0.192	0.172	0.168	0.257	0.195	0.181	0.224	0.205	0.237	0.185	0.177
EINPP	0.119	0.062		0.190	0.174	0.053	0.182	0.126	0.279	0.193	0.155	0.191	0.182	0.258	0.149	0.133
FNWR	0.145	0.057	0.077		0.194	0.272	0.258	0.251	0.377	0.302	0.231	0.253	0.236	0.292	0.266	0.209
NBR	0.124	0.085	0.073	0.08		0.269	0.240	0.230	0.320	0.226	0.19	0.208	0.211	0.255	0.195	0.187
PM	0.159	0.079	0.025	0.112	0.114		0.218	0.141	0.320	0.251	0.21	0.236	0.244	0.341	0.189	0.188
WMWR	0.126	0.079	0.084	0.113	0.108	0.102		0.090	0.303	0.204	0.218	0.246	0.282	0.289	0.199	0.212
YNP	0.109	0.067	0.054	0.100	0.095	0.064	0.044		0.296	0.159	0.196	0.253	0.260	0.282	0.163	0.185
EINPW	0.154	0.099	0.114	0.146	0.130	0.135	0.135	0.122		0.163	0.055	0.078	0.070	0.053	0.069	0.088
MBS	0.123	0.092	0.092	0.134	0.106	0.119	0.102	0.078	0.081		0.074	0.116	0.099	0.107	0.078	0.084
WBNP	0.104	0.069	0.065	0.092	0.079	0.091	0.098	0.082	0.025	0.081						
GR	0.135	0.085	0.080	0.101	0.087	0.102	0.110	0.104	0.036	0.059			0.038	0.063	0.045	0.043
LB	0.118	0.081	0.077	0.097	0.090	0.106	0.125	0.108	0.033	0.051		0.017		0.036	0.045	0.036
NL	0.128	0.095	0.109	0.120	0.120	0.144	0.131	0.119	0.026	0.055		0.030	0.017		0.061	0.063
PL	0.096	0.070	0.062	0.104	0.081	0.082	0.091	0.069	0.032	0.041		0.020	0.021	0.029		0.035
SW	0.107	0.069	0.057	0.085	0.079	0.083	0.097	0.079	0.040	0.044		0.020	0.017	0.030	0.016	

Table 2-8B. D_{LR} results are above the diagonal and $(\delta\mu)^2$ results are below the diagonal.

	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP	GR	LB	NL	PL	SW
AISP		6.63	6.5	8.73	6.85	7.42	7.22	6.29	8.23	6.59	5.41	6.14	5.15	6.43	4.99	5.15
CSP	10.77		3.91	3.61	5.47	4.25	4.73	4.03	6.58	5.06	3.80	3.93	3.42	5.06	4.02	3.71
EINPP	12.43	7.27		5.14	4.25	0.46	5.89	3.46	8.32	5.85	4.55	4.54	4.57	6.75	4.48	3.61
FNWR	10.57	3.70	6.10		5.57	6.58	7.42	6.26	10.38	7.79	5.83	5.43	5.11	7.58	6.89	4.97
NBR	21.70	6.80	12.69	8.80		5.59	6.53	5.54	8.73	6.52	5.11	4.48	4.62	7.07	5.27	4.52
PM	13.84	10.21	3.68	10.05	15.99		6.36	3.40	8.34	6.02	4.74	4.86	4.95	7.28	4.23	3.95
WMWR	2.78	7.01	5.35	8.06	17.55	8.84		3.60	8.17	6.62	5.84	3.95	6.46	6.75	5.64	5.91
YNP	11.03	5.10	7.94	8.83	4.78	7.92	8.28		7.35	5.03	4.66	5.00	5.22	6.47	4.00	4.26
EINPW	20.72	11.38	14.80	13.47	9.48	10.76	17.81	10.66		3.53	1.48	1.15	1.21	1.08	1.52	2.06
MBS	9.62	6.00	11.53	8.11	4.99	13.15	8.92	5.11	5.64		1.45	1.31	1.11	1.85	1.70	1.55
WBNP	13.74	7.09	10.86	8.21	7.72	7.70	12.34	7.44	1.45	3.28						
GR	18.48	10.28	15.05	13.37	10.86	9.59	17.00	10.15	0.72	6.09			0	0	0	0
LB	13.23	6.92	11.46	6.15	7.12	8.44	13.29	7.16	2.96	4.11		2.31		0	0.08	0
NL	20.57	9.85	16.77	10.45	6.90	14.88	19.19	11.41	1.49	4.26		2.25	2.01		0.96	0.77
PL	14.43	6.70	10.33	8.48	7.39	7.88	11.97	7.50	1.51	3.07		1.35	1.75	1.90		0.20
SW	10.55	8.01	9.10	9.14	11.14	5.13	9.46	7.36	4.04	4.68		2.82	2.69	5.39	1.29	

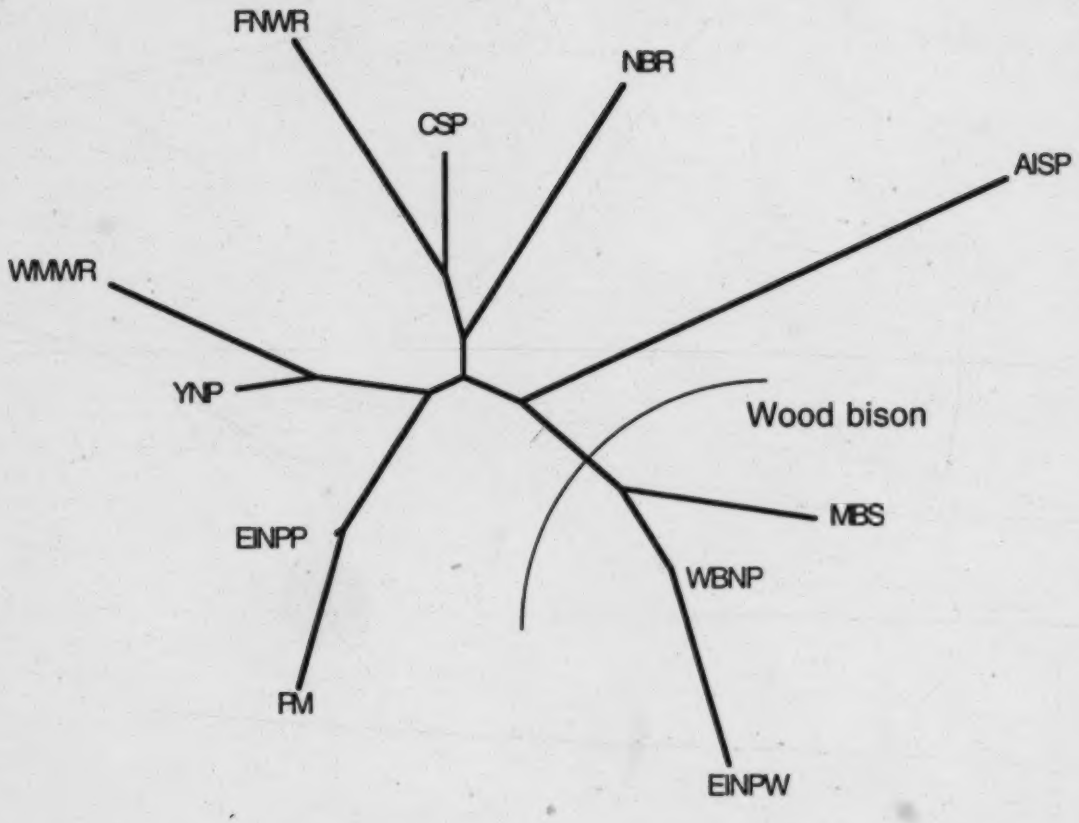

Figure 2-1. Nei's minimum unrooted tree using the Fitch and Margoliash method. Abbreviations can be found in Table 2-1.

Chapter 3

*Conservation of Wood Bison (*Bison bison athabascae*) in Canada: a Genetic Perspective*

Introduction

Wood bison (*Bison bison athabascae*) were once one of the most prominent megaherbivores in northern North America. However, like their plains bison counterparts, they underwent a precipitous decline in numbers in the late 1800s, to the point where a single population existed in the region currently designated Wood Buffalo National Park. Unfortunately, bison in this park became infected with brucellosis and tuberculosis after the addition of plains bison to the region. A recommendation put forth by a Federal Environmental Review Panel (1990) suggests that Wood Buffalo National Park be depopulated and replaced with salvaged disease-free animals. To date, there are three main wood bison populations salvaged from Wood Buffalo National Park. We determined the genetic diversity in the newest of these salvage populations, the Hook Lake Wood Bison Recovery Project, and compared it to the other wood bison populations to determine if this proposed depopulation event would result in an irreplaceable loss of wood bison genetic diversity. The effect of number of founders and time since population establishment on the diversity of the salvaged populations was also examined. As the Hook Lake Wood Bison Recovery Project was recently salvaged, we were given a unique opportunity to observe the loss of diversity in this population over a single generation due to male differential reproductive success.

The original range of wood bison (*Bison bison athabascae)* included the boreal forest areas of northern Canada and Alaska (Van Zyll de Jong 1986, Guthrie 1990,

Stephenson et al. 2001). While the number of wood bison existing in this region is unknown, Soper (1941) estimated that the carrying capacity of their range was well over 100 000 in 1800. Wood bison were extirpated from most of their range in the late 1800s, and only about 250 animals remained at the turn of the last century (Soper 1941). Most of these animals existed in the region currently designated as Wood Buffalo National Park in Alberta and the Northwest Territories, Canada. Wood bison are currently considered threatened in Canada (COSEWIC 1998).

The first effort to conserve wood bison occurred in 1877, with the passing of the Buffalo Protection Act (Hewitt 1921). In 1922, when wood bison numbers had increased to approximately 1500, Wood Buffalo National Park was established to protect the remaining wood bison (Soper 1941). At about this time, the plains bison (*Bison bison bison*) population at Buffalo Park in Wainwright, Alberta was experiencing overcrowding. Despite the objections of biologists about the risk of hybridization between these groups of animals (see Harper 1925, Saunders 1925), 6673 young plains bison, infected with bovine brucellosis and tuberculosis, were shipped from Buffalo Park to Wood Buffalo National Park between 1925 and 1928 (Ogilvie 1979). However, perhaps as many as 50% of these animals were not successfully introduced to Wood Buffalo National Park (see Carbyn et al. 1993). Also, as the introduced male plains bison were young, it is unlikely that the larger, mature wood bison males allowed many of them to breed (Carbyn et al. 1993).

Genetic evidence suggests that the plains bison released into Wood Buffalo National Park hybridized with the wood bison (Polziehn et al. 1996, Wilson and Strobeck 1999a). Some people feel that this hybridization has led to the loss of wood bison as a subspecies (Geist 1991, Polziehn et al. 1996), while others argue that there is still enough morphological differentiation between populations of wood and plains bison to warrant subspecific status (Van Zyll de Jong et al. 1995). Tuberculosis and brucellosis are now

prevalent in the bison at Wood Buffalo National Park (Joly and Messier 2001). The number of bison in Wood Buffalo National Park has dropped from about 10 000 animals in 1970 to 2200. This large decline in bison numbers has been hypothesized to be due to the presence of the two introduced diseases, and their combined effects on wolf predation (Gates 1993, Messier and Blyth 1996, Joly and Messier 2001, but see McCormack 1992, Carbyn et al. 1993 for an alternate explanation).

There have been three attempts to salvage tuberculosis- and brucellosis-free wood bison from Wood Buffalo National Park and surrounding regions. In 1963, sixteen wood bison were used to found a herd in the Mackenzie Bison Sanctuary, Northwest Territories. These animals had been captured in northwestern Wood Buffalo National Park from a region thought to contain wood bison that had not hybridized with the introduced plains bison, and were determined to be disease-free. Independent studies of morphological characters and mitochondrial (mtDNA) haplotypes have since shown that these bison were not pure, but had already hybridized (Van Zyll de Jong et al. 1995, Polziehn et al. 1996).

In 1965, a second salvage was attempted. Twenty-one animals were shipped from northern Wood Buffalo National Park to Elk Island National Park, Alberta. To eliminate tuberculosis from this population, the original stock was extirpated and their 11 calves used as founders. The Elk Island National Park wood bison population is of great conservation significance as it is being used for the establishment of new populations in Canada. Both the Mackenzie Bison Sanctuary and the Elk Island National Park wood bison populations contain less genetic variation than that found in Wood Buffalo National Park, likely due to the small number of individuals used to found these populations (Wilson and Strobeck 1999a). In 1990, a Federal Environmental Assessment Panel recommended that Wood Buffalo National Park be depopulated, and replaced with animals primarily from Elk Island National Park to ensure that diseased animals did not

migrate into the region currently inhabited by the Mackenzie Bison Sanctuary population. This plan has yet to be implemented. Had this occurred, much of the genetic variation found in the Wood Buffalo National Park population would have been lost, as it was not represented in any of the other wood bison populations.

A third salvage attempt was carried out from the Hook Lake herd (hereafter referred to as the Hook Lake Region) west of Wood Buffalo National Park between 1996 and 1998. About twenty calves were captured from the Hook Lake Region in each of these years, and tested for tuberculosis and brucellosis. The cohorts captured in 1996 and 1997 have also since produced offspring. Bison have been observed moving between the Hook Lake Region and the Wood Buffalo National Park population, however the amount of gene flow between these regions is unknown. This study examines the diversity in the founders of the Hook Lake Wood Bison Recovery Project (hereafter referred to as the captive Hook Lake population), and compares its success as a genetic salvage operation with the two prior attempts. An effective population size of 50 would be required to obtain 99% of the genetic variation in a population. In total, 58 calves were used to found the captive Hook Lake population. Due to the large number of founders used for the captive Hook Lake population and short amount of time genetic drift could have altered the allele frequencies in this population, we expect it to be the most variable of the salvaged populations, and the most genetically similar to Wood Buffalo National Park.

There should be two primary goals for any genetic salvage operation. First, the salvaged population should contain a representative sample of the genetic variation present in the original population. Secondly, excessive amounts of this genetic variation should not be lost from the salvaged population through time. Genetic variation has a greater chance of being lost from small populations through the processes of differential reproduction, inbreeding, and genetic drift, so the salvaged population will be particularly

susceptible to the loss of genetic variation shortly after it has been founded, before it has had a chance to increase in size (Frankel and Soule 1981, Shaffer 1987, Brussard and Gilpin 1989). Also, in order to maximize fitness of any new population, it should be established from the most genetically variable of the available potential founder populations (see for ex. Hedrick et al. 2001). Therefore, the amount of genetic variation present in the salvaged populations could be used to determine which, if any, should be used to found future wood bison populations.

Microsatellites are highly polymorphic nuclear markers (Tautz 1989, Weber and May 1989) that have proven useful in examining the genetic variation and structure of genetically depauperate populations (for ex. Houlden et al. 1996, Goossens et al. 2001). They have also been able to establish paternity in bison (Mommens et al. 1998, Schnabel et al. 2000). We used 11 microsatellite loci to compare the amount of genetic variation in the captive Hook Lake and Hook Lake Region populations with Wood Buffalo National Park and the other two salvaged populations (previously obtained in Wilson and Strobeck 1999a). We also established the paternity of the calves born in the captive Hook Lake population to determine the reproductive success of the males in this population, and to examine the amount of variation that was absent in the calves but present in their parent generation.

Materials and Methods

Laboratory Methods

DNA was isolated from blood or tissue samples of all the bison used to found the captive Hook Lake population, their 33 calves, and 26 animals from the Hook Lake Region using

a QIAamp® Tissue Extraction Kit. The following microsatellite loci were used in this study: RT9, RT24, and RT27 (Wilson et al. 1997), BOVFSH (Moore et al. 1992), Eth121 (Steffen et al. 1993), BM143, BM1225, BM2830, BM4513, and BMC1222 (Bishop et al. 1994), and RT29 (Wilson and Strobeck 1999a). These loci are the same as those used in a survey of genetic variation in North American bison populations by Wilson and Strobeck (1999a), allowing direct comparison of the two sets of results. When these loci were not capable of excluding all but one male as potential fathers, some of BM4440, BM723, BM5004, BM3507, BM1819, BM1824 (Bishop et al. 1994), TGLA57 (Barendse et al. 1993), CSSM022 (Barendse et al. 1994), Eth152 (Steffen et al. 1993), and BBJ24 (Wilson and Strobeck 1999b) were also amplified. One primer from each of these sets was fluorescently labelled with either a FAM, HEX or TET dye group. The unlabelled primer for loci TGLA57, CSSM022 and BM1824 were tailed as in Brownstein et al. (1996) to promote adenylation of the PCR product. Loci were multiplexed (amplified in the same reaction during PCR) when possible. Each PCR reaction contained 2.5 mM $MgCl_2$ and PCR buffer (10 mM Tris buffer, pH 8.8, 0.1% Triton X100, 50 mM KCl, 0.16 mg/mL BSA). Concentrations of all other contents of the PCR reactions, and multiplexes performed, can be found in Table 3-1. *Taq* polymerase was purified as described in Engelke et al. (1990). PCR cycling conditions were: 1 min at 94°C; 3 cycles of 30 s at 94°C, 20 s at 54°C, 5 s at 72°C; 33 cycles of 15 s at 94°C, 20 s at 54°C, 1 s at 72°C; 30 min extension at 72°C. PCR reactions were performed on an ABI 9600 thermal cycler and electrophoresed on an ABI 373A or ABI 377 DNA Sequencer.

Data Analysis

All loci examined in the Hook Lake Region and captive Hook Lake populations were tested for heterozygote deficiency with GENEPOP v3.1d, which uses a Markov chain

algorithm (Raymond and Rouset 1995). Linkage disequilibrium between loci was also examined using GENEPOP v3.1d software. Unless mentioned, all results reported for the captive Hook Lake population did not include the calves born in that population.

Three methods were used to examine the amount of genetic variation within the populations: mean number of alleles, average unbiased expected heterozygosity (Nei and Roychoudhury 1974) and the overall unbiased probability of identity (Paetkau et al. 1998). Probability of identity values were compared across populations over all loci using Wilcoxon's signed-ranks test (Sokal and Rohlf 1995). The following Wilcoxon signed-rank comparisons were performed to test for significant differences in variability between populations: the captive Hook Lake founders to all other wood bison populations and their calves, Wood Buffalo National Park to all other wood bison populations, and Mackenzie Bison Sanctuary to Elk Island National Park. Probability of identity was chosen because it is not as dependent on sample size as the mean number of alleles is, and was found to correlate more strongly with mean number of founders than heterozygosity did (Wilson and Strobeck 1999a). Genetic variation values for the Wood Buffalo National Park, Mackenzie Bison Sanctuary and Elk Island National Park populations were taken from Wilson and Strobeck (1999a).

The genetic distinctiveness of populations was examined with the G-test for heterogeneity and the assignment test. The G-test was used to compare allele distributions across all wood bison populations, each cohort of animals used to found the captive Hook Lake population, and the calves born in this population (Sokal and Rohlf 1995). The assignment test compares each individual's genotype to the allele frequencies of all populations, and assigns them to the population the genotype is most likely to occur in (Paetkau et al. 1995). All wood bison genotyped in this study and in Wilson and Strobeck (1999a) were compared to all wood and plains bison populations from the two studies with the assignment test. The calves from the captive Hook Lake population were

examined with the assignment test, but were not included in the allele frequency calculations. A program for calculating the assignment test is available at http://www.biology.ualberta.ca/jbrzusto/Doh.php. Note that the assignment test was calculated differently than in Wilson and Strobeck (1999a). In the prior paper, the genotype of the individual examined was added to the allele frequencies of each population, to allow for sampling bias. Allele frequencies of 0 were assigned a value of 0.01. In this paper, the individual's genotype was instead removed from its source population. All allele frequencies were adjusted to avoid frequencies of 0 as in Titterington et al. (1981). These changes resulted in some minor differences in assignments between populations.

The genetic relatedness among all wood bison populations was examined using two genetic distance measures: D_S (Nei 1973) and D_{LR} (Paetkau et al. 1997). These measures were chosen because they are known to be reliable (Takezaki and Nei 1996, Paetkau et al. 1997), but are calculated in different ways. D_S makes use of differences in allele frequencies between populations, whereas D_{LR} is based the number of misassignments from the assignment test. Genetic distances were calculated using programs available at http://www.biology.ualberta.ca/jbrzusto/GeneDist.php. D_{LR} values differed from those calculated from Wilson and Strobeck (1999a) because of the changes in the calculation of the assignment test described above. Neighbour-joining (Saitou and Nei 1987) unrooted trees were designed from the genetic distance data using PHYLIP 3.573 (Felsenstein 1995).

The cohorts used to found the captive Hook Lake population were examined with RELATEDNESS 5.0.6 (Goodnight and Queller 2000) to determine if the individuals within them are more closely related than expected by chance. If the three cohorts used to found the captive Hook Lake population contain a number of paternal half sibs as a result of unequal male mating success, they will be more related to each other than

expected. Allele frequencies from all of the founders in this population were used to derive relatedness coefficient (*R*) values between all pairs of individuals. The mean within-cohort pairwise comparisons were then compared to the mean among-cohort comparisons.

Individual distances between wood bison from Wood Buffalo National Park, the Hook Lake Region, and the captive Hook Lake population were calculated using shared alleles (Bowcock et al. 1994) and 1-*R* (obtained from RELATEDNESS 5.0.6) to check for any structure within these groups. There are five different subpopulations in Wood Buffalo National Park - Garden River, Sweetgrass, Needle Lake, Pine Lake, and Little Buffalo - with an unknown amount of gene flow between them. If there is structure within this population, then all distinct regions should be taken into account during a genetic salvage operation. Structure within the Hook Lake Region or the captive Hook Lake populations would suggest that these populations are distinct from Wood Buffalo National Park. Majority-rule consensus neighbour-joining trees (Saitou and Nei 1987) jackknifed over all loci were designed using PHYLIP 3.573 (Felsenstein 1995).

Paternity of the calves was calculated in two ways. Exclusion was used, where paternity was assigned to the only male that matched the calf's paternally contributed alleles at all loci. When maternity was unknown, mothers were first assigned to the offspring by determining which female contained at least one of the alleles present in the calf's genotype at all loci. Maternity was known for all but seven of the 33 calves born in the captive Hook Lake population in 1999 and 2000. These seven mothers were not kept in isolation, so maternity could not be definitively established through observation. Exclusion does not allow for the possibility of a mismatch between the parent and offspring genotype because of a mutation or human error, and is only useful in cases where most of the potential parents have been sampled. CERVUS 1.0 (Marshall et al. 1998), which uses a likelihood method, was also used to assign paternity. The ability of

CERVUS 1.0 to establish parentage in large, natural populations has been established by Slate et al. (2000). When both parents were unknown, parentage was assigned in a stepwise manner, with maternity first being assigned to the offspring. The CERVUS 1.0 simulations required for parentage assignment were performed with the following parameters: 1000 cycles, 10 potential parents, 100% of candidate parents sampled, 100% of loci typed, and 1% of all loci mistyped. The typing error rate was an overestimate, based on the number of mismatches found between known mothers and offspring.

Results

None of the loci were deficient in heterozygotes in either the captive Hook Lake or the Hook Lake Region populations ($p>0.05$). Also, none of the 90 locus pairwise tests for linkage disequilibrium were found to be significant in either population, when the Dunn-Sidak experiment-wise error rate was used ($p>0.05$, Sokal and Rolf 1995).

The genetic variation in the captive Hook Lake founders, the calves born in this population, and the Hook Lake Region population are in Table 3-2, along with prior results for the wood bison populations of Elk Island National Park, Mackenzie Bison Sanctuary and Wood Buffalo National Park using the same 11 microsatellite loci (Wilson and Strobeck 1999a). Two of the variability measures – mean number of alleles and overall unbiased probability of identity – show that the captive Hook Lake population was more variable than both Elk Island National Park and Mackenzie Bison Sanctuary. The mean heterozygosity in Elk Island National Park was greater than that in the captive Hook Lake population, although these values were similar. Calves born to the captive Hook Lake population were less variable than the founders of this population with all

measures. Genetic variation was greatest in Wood Buffalo National Park with all measures.

Probability of identity values for each locus-population combination can be found in Table 3-3. Using the Wilcoxon signed rank test, the captive Hook Lake population was found to be significantly more variable than the calves born in this population ($P<0.005$), while it was significantly less variable than Wood Buffalo National Park ($P<0.05$). Mackenzie Bison Sanctuary was also less variable than Wood Buffalo National Park ($P<0.05$). All other comparisons were non-significant. Similar results were found when the heterozygosity values for each locus were compared across populations (data not shown).

The G-test for heterogeneity did not detect significant differences between any of the three cohorts used to found the captive Hook Lake population ($P>0.10$). Therefore, the cohorts were treated as a single group for all analyses. The captive Hook Lake population was not significantly different from the Hook Lake Region population ($P>0.05$) but it was from all other wood bison populations ($P<0.001$). The Hook Lake Region individuals were not significantly different from Wood Buffalo National Park ($P>0.10$). Calves born in the captive Hook Lake population were significantly different from the founders of this population ($P<0.05$).

The assignment test was performed using all wood bison genotyped in this study and the Wilson and Strobeck (1999a) study (Table 3-4). Many of the captive Hook Lake, Hook Lake Region, and Wood Buffalo National Park individuals were misassigned across these populations, while Mackenzie Bison Sanctuary and Elk Island National Park were more insular. All of the individuals from the captive Hook Lake population were assigned to wood bison populations, although 55% were assigned to different wood bison populations (31% of these to the Hook Lake Region). Only 19% of the Hook Lake Region population was correctly assigned to itself, with most misassignments either to

the captive Hook Lake population (46%) or Wood Buffalo National Park (23%). Most of the calves born to the captive Hook Lake population were assigned to that population (76%). Wood Buffalo National Park animals were also frequently misassigned. Forty-one percent of these animals were assigned to their source population, while 26% were assigned to the captive Hook Lake population and 17% to the Hook Lake Region population. Only four wood bison (three from Wood Buffalo National Park and one from the Hook Lake Region) were assigned to plains bison populations.

D_S and D_{LR} distances between all wood and plains bison populations from this study and Wilson and Strobeck (1999a) can be found in Table 3-5. With both measures, the genetic distances within each subspecies tended to be smaller than those between subspecies. The smallest genetic distances were between the Hook Lake Region population, the captive Hook Lake population, and Wood Buffalo National Park. Elk Island National Park and Mackenzie Bison Sanctuary were the most distantly related of the wood bison populations. The D_S unrooted tree designed using the neighbour-joining method (Saitou and Nei 1987) is given in Figure 3-1. The D_{LR} unrooted tree resembles this one, except the (EINPP-PM) branch joins with the (NBR, FNWR, CSP) clade instead of the (YNP-WMWR) clade, and there are some minor differences in branch lengths (not shown). Again, the captive Hook Lake, the Hook Lake Region and Wood Buffalo National Park populations form a closer group than any of the other populations. All of the wood bison cluster together on this tree.

There were no differences in genetic relatedness (pairwise *R*) between any of the captive Hook Lake population cohorts and the among-cohort *R* values (Table 3-6). Two pairs of individuals: 44 and 124 (from 1996 and 1998), and 47 and 80 (from 1996 and 1997) had very high *R* values (0.780 and 0.763, respectively), suggesting that they may be related at least at the half sib level (theoretical *R* value for second order relatives is 0.25).

Pairwise shared allele and (1-*R*) distances were calculated between 198 wood bison from Wood Buffalo National Park, Hook Lake Region, and the captive Hook Lake population (including calves). Both of the consensus trees designed using these distances revealed very little structure between the wood bison from any of these populations (data not shown). When the (1-*R*) values were jackknifed over all loci only nine groups, consisting of two or three individuals, were found in every tree. Ten similarly sized groups were found from the shared allele data. Four of these groups on the (1-*R*) tree and seven of the groups on the shared allele tree contained animals from a mixture of subpopulations, while two groups on each tree contained mother-offspring pairs from the captive Hook Lake population. Seventy-nine and 55 groups were significant at the 50% level for the (1-*R*) and shared allele consensus trees, respectively. Only nine of these groups consisted of more than three individuals. Forty-eight of these groups were in common between both trees, suggesting that neither of these methods is better able to detect structure using our data.

Results of the paternity tests can be found in Table 3-7. During the 1998 breeding season (which produced the calves born in 1999), each of the three cohorts was kept separately. At this time, only the 1996 cohort was old enough to produce viable offspring, so there were only five possible breeding males and 13 females, who produced nine calves. In the 1999 breeding season, the 1996 and 1997 cohorts were mixed together, resulting in a breeding population of 29 females and nine males, which produced 24 offspring. The 1997 males were included as potential fathers in the analyses, even though it is unlikely that they were successful breeders, as they were less mature than the dominant 1996 males. When exclusion was used, maternity could be assigned to six of the seven calves whose mother was unknown. For the seventh calf, two females could not be eliminated as parents. However, there was only one possible mother-father pairing which could explain the calf's genotype, so maternity was assigned

in this manner. On average, the next closest female was excluded at one locus. Every calf but one was assigned a single father using exclusion, although in two cases more than the 11 initial loci needed to be examined. For the calf where parentage assignment was not possible, two males could not be excluded as a father: a 1996 male and a 1997 male, for which DNA was not available to examine more loci. As the 1996 males were clearly dominant to their younger counterparts, and no other 1997 males reproduced successfully, paternity for this calf was assigned to the 1996 male. The next closest males were excluded at an average of 2.75 loci for the 1996 cohort and 1.6 loci for the 1997 cohort. Nineteen and 17 loci were used to determine paternity in the two cases where the 11 loci were unable to eliminate all males but the true father.

Since most of the individuals from the captive Hook Lake population were only genotyped at 11 loci, no other loci were included in the CERVUS 1.0 analysis. CERVUS 1.0 was first used to assign maternity to the seven calves whose mothers were unknown. In six cases, a mother was assigned at the 95% confidence level, and maternity was assigned to the other calf at the 80% confidence level. This was not the same calf where two females could not be eliminated as mothers using exclusion. Even though extra loci were required for exclusion to assign fathers to some of the calves, paternities were assigned to every calf at the 95% level with CERVUS 1.0, even when males from the 1996 and 1997 cohorts were included as potential fathers. In every case, the same father was assigned using both methods. For the 1996 calf where exclusion was impossible with 11 loci, four males matched at all 11 loci. CERVUS 1.0 still assigned paternity to this calf at the 95% confidence level. The CERVUS 1.0 and exclusion parentage assignments agreed in all cases.

Discussion

Measures of genetic variation reveal that Wood Buffalo National Park is the most diverse wood bison population, followed by the Hook Lake Region and captive Hook Lake populations. The latter population has a lower mean heterozygosity than Elk Island National Park, but these values may not be significantly different (Table 3-2). Since Elk Island National Park is one of the least variable wood bison populations, it may not be the best genetic choice for founding new wood bison populations. Animals from the captive Hook Lake population, or a mixture of animals from the two populations, would give a better representation of the genetic variation present in Wood Buffalo National Park. Comparisons to some other North American ungulates show that wood bison have intermediate levels of genetic variation. Genetic diversity in wood bison is higher than in moose (Broders et al. 1999), similar to wapiti (Polziehn et al. 2000) and plains bison (Wilson and Strobeck 1999a), and lower than caribou (Zittlau et al. 2000). This suggests that, despite the population bottleneck undergone by wood bison, there has not been a large negative effect on the genetic diversity of this group.

Both the G-test and the Wilcoxon signed-rank test show that the captive Hook Lake population is significantly different (and less variable) than Wood Buffalo National Park, despite the non-significant differences in allele frequencies between the captive Hook Lake and Hook Lake Region populations, and the Hook Lake Region and Wood Buffalo National Park populations. The founders of the captive Hook Lake population were captured in the Hook Lake Region, which is adjacent to Wood Buffalo National Park. There must be a slight, non-significant difference in allele frequencies between the captive Hook Lake population and the Hook Lake Region population, and this region and Wood Buffalo National Park, so that when taken together, the captive Hook Lake population is significantly different than Wood Buffalo National Park. These results

suggest that there is significant gene flow between the Hook Lake Region and Wood Buffalo National Park, and that the individuals in the captive Hook Lake population contain a representative amount of the genetic variation present in the Hook Lake Region population, but are slightly less variable than Wood Buffalo National Park animals.

When probability of identity values were compared using the Wilcoxon signed-rank test, Elk Island National Park was not significantly less variable than Wood Buffalo National Park, whereas the captive Hook Lake population was less variable than Wood Buffalo National Park. This occurred despite the fact that the overall probability of identity value for the captive Hook Lake population was lower (i.e. variability was higher) than that for Elk Island National Park. The reason for this seems to be that the probability of identity values for the captive Hook Lake population are slightly but consistently higher than the Wood Buffalo National Park values, whereas the Elk Island National Park values are often higher than both of these populations, but vary more around them. Presumably, this has occurred because of the length of time that the Elk Island National Park population has had to diverge from its founding population due to genetic drift. The Wilcoxon signed-rank test may not be able to detect differences in genetic variation in populations that have been separated for more than a few generations.

The large number of cross assignments in the assignment test, small genetic distances between populations, and clumping on the neighbour-joining tree designed using the genetic distance values all suggest that there is little differentiation between Wood Buffalo National Park, the Hook Lake Region, and the captive Hook Lake population. These populations are more similar to each other than any of the other wood or plains bison populations described by Wilson and Strobeck (1999a). Genetic distances between wood bison populations are generally as small as, or smaller than, those found between populations of wapiti, another North American ungulate, that are known to have some level of gene flow between them (Polziehn et al. 2000). The distances between the

Hook Lake Region, the captive Hook Lake, and Wood Buffalo National Park populations are smaller than any values observed in Polziehn et al. (2000). The distinctness of wood bison is supported in this study since between-subspecies distances are generally larger than the within-subspecies distances, wood bison group together on the neighbour-joining tree, and only 4 of 262 (or 1.5%) of the wood bison cross-assigned to plains bison populations, despite the hybridization between wood and plains bison in Wood Buffalo National Park. This suggests that there are genetic differences between wood and plains bison that should be maintained by continuing to manage these groups separately.

If the captive Hook Lake population founders were close relatives, then the genetic variation in this population would not be as high as expected, and the chance of inbreeding would be increased. Relatives would most likely occur within cohorts, as a few dominant males could produce most of the offspring in a season. In a study of Cunningham's skinks (*Egernia cunninghami*), Stow et al. (2001) showed that differences in mean pairwise relatedness comparisons were useful in detecting differences in genotypic similarity among groups. As the mean pairwise relatedness (R) values within the captive Hook Lake population cohorts were not significantly different than those among cohorts, there is no evidence that the individuals within cohorts are more related to each other than expected by chance. The lack of abnormally large R values among any of the captive Hook Lake population founders also suggests that there are few closely related individuals within this population.

Very few relationships between groups of Wood Buffalo National Park, captive Hook Lake population, and Hook Lake Region bison were significant when either the shared alleles or (1-R) individual distances were jackknifed across loci. The relationships that were significant were often a mixture of animals from different regions. This result is important for two reasons. First, it suggests that there is little population substructure within Wood Buffalo National Park. This is expected given the large movements

observed by some of the bison in the park (Wilson and Milne 1992), and fluidity of other bison herds (Lott and Minta 1983, Van Vuren 1983). Gene flow between all subpopulations must be fairly extensive, and the plains bison genetic material introduced to this area from 1925 to 1928 must have spread throughout the park. All regions should contain approximately equal amounts of plains bison genetic material. Also, it is not necessary to genetically salvage individuals from various regions of Wood Buffalo National Park in order to obtain a representative amount of the genetic material present in this population. Second, the lack of distinction between the founders of the captive Hook Lake population and Wood Buffalo National Park suggests there are few genetic differences between these populations. If the captive Hook Lake population individuals were distinct from the Wood Buffalo National Park population, they would clump together on the neighbour-joining tree, which was not observed.

Both exclusion and likelihood (applied with the CERVUS 1.0 program) were able to assign parentage for the calves in the captive Hook Lake population in all of the unknown cases. These methods were in agreement for each parentage assignment. The success of the 11 microsatellite loci in establishing paternity can be attributed to three reasons: i) we had sampled all potential fathers, ii) there were few males that needed to be eliminated as potential fathers, and iii) in most cases maternity was known. Male 37, observed to be the dominant male in the herd (J. Nishi pers. comm.), did not have the highest overall reproductive success (Table 3-7). However, 37 and 44 seemed to be the highest ranking males in this herd and their combined reproductive success accounted for over 90% of the offspring born during the two year period. Dominance may not be a good indicator of reproductive success, since male plains bison are known to lose their dominance ranking as they become exhausted during the breeding season (Lott 1979), multiple matings do occur (Lott 1981), and copulation does not necessarily coincide with ovulation (Komers et al. 1994). Another possibility is that dominance in this herd can be

used to predict mating success but not reproductive success. Male 37 may have lower quality or quantity semen, which has been linked with reproductive success in cattle (for review see Foote 1989).

The ability of male wood bison to dominate reproduction seems to decline as the number of females increases. In 2000, the number of mature females increased to 29 from 13 the previous year, and the number of successful males increased from two to four. Males are likely less able to exclude potential rivals from reproducing as the number of mature females rises. If dominant males are better able to control breeding in small populations, this could have had an effect on the genetic variation found in Elk Island National Park and Mackenzie Bison Sanctuary. Both of these populations started from less than 20 individuals, so few males were likely able to breed any year. This could have exacerbated the effects of genetic drift in these populations.

The calves born in the captive Hook Lake population were significantly less variable than the founders of this population, and their allele frequencies were significantly different. This is likely due to the small number of reproductively successful males, which lowers the effective size of a population. If the number of breeding males continues to be small, genetic variation present in this population - successfully salvaged from the wild - will be lost through genetic drift. However, the number of breeding males in this population will increase as the number of mature females increases, if current trends continue. Mating success in the captive Hook Lake population should continue to be monitored to ensure that an excessive amount of genetic variation is not quickly lost from this population due to differences in male reproductive success.

The fact that the Wood Buffalo National Park population is more genetically variable than any of the other wood bison populations suggests that more genetic salvage attempts should be undertaken if Wood Buffalo National Park is to be depopulated and

replaced with animals from other areas (Federal Environmental Assessment Review Panel 1990). Failure to do this would result in the loss of the genetic diversity that is unique to the Wood Buffalo National Park population. The number of individuals required in future salvage operations depends on the amount of variability loss that is deemed acceptable. The effective size (N_e) of a salvaged population required to obtain a given amount of the diversity present in the Wood Buffalo National Park population can be calculated with formula [1]

[1] $$H_1 = (1 - 1/2N_e)\, H_0$$

where the ratio H_1/H_0 is the proportion of the genetic variation present in the Wood Buffalo National Park population desired in the salvaged population. To avoid excessive loss of diversity, a reasonable goal may be to attempt to obtain approximately 99% of the genetic variation present in the Wood Buffalo National Park population. This would require an effective population size of 50.

The effect of an unbalanced sex ratio in a population on N_e can be calculated using formula [2]

[2] $$N_e = 4\,(N_f \times N_m) / (N_f + N_m)$$

where N_f and N_m are the number of females and males in the population, respectively. As bison are polygynous and few males breed every year (Chapter 4, this thesis), a reasonable sex ratio for the founded herd may be 25% males and 75% females. If this is the case, then about 67 animals should be used to start a salvaged population.

Genetic drift will begin to lower the genetic diversity in any new salvaged population (Frankel and Soule 1981, Shaffer 1987, Brussard and Gilpin 1989). However,

it is unlikely that the same genetic variation will be lost across salvaged populations. Therefore, at least two more salvage operations from Wood Buffalo National Park of about 67 individuals should be performed to ensure that a suitable amount of the genetic variation of the former population is sampled and not lost through the process of genetic drift. This way we can be fairly confident that the genetic variation in the Wood Buffalo National Park population is sampled and maintained even if this region is depopulated.

Summary

Since the addition of brucellosis- and tuberculosis-infected plains bison to Wood Buffalo National Park, there has been a desire to establish a disease-free herd of wood bison. The previous two attempts - the herds started in Elk Island National Park and Mackenzie Bison Sanctuary - were only partly successful. While brucellosis and tuberculosis are not present in these populations, the genetic variation therein is not representative of that found in Wood Buffalo National Park. The recent establishment of the captive Hook Lake population has come closer to this goal. This population is the most genetically similar to Wood Buffalo National Park of the three salvage attempts. However, it is still significantly less variable than the Wood Buffalo National Park population. If Wood Buffalo National Park is to be depopulated and replaced with wood bison from other regions (Federal Environmental Assessment Review Panel 1990), at least two more salvage attempts should be undertaken to ensure that the genetic variation present in this population is not lost forever. Also, mating success in populations like the captive Hook Lake population should be managed to ensure that excessive amounts of the genetic variation contained within them is not lost through the processes of inbreeding and genetic drift.

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Table 3-1. Remainder of the contents and amounts for the PCR reactions, in μM unless stated. Loci are amplified in the following reaction mixes: BM143 (locus 1A), BM2830 (1B), BM1225 (1C), RT29 (2A), BMC1222 (2B), BM4513 (2C), RT27 (3A), RT24 (3B), RT9 (3C), BM4440 (4A), BM723 (4B), BM1824 (4C), BM5004 (5A), BBJ24 (5B), TGLA57 (5C), Eth121 (6), BOVFSH (6), CSSM022 (6), BM3507 (7), Eth152 (8), and BM1819 (9).

	1	2	3	4	5	6	7	8	9
Each Primer A	0.19	0.19	0.18	0.15	0.19	0.16	0.16	0.16	0.16
Each Primer B	0.19	0.19	0.16	0.16	0.19	-	-	-	-
Each Primer C	0.17	0.13	0.16	0.21	0.16	-	-	-	-
dNTPs	160	160	160	160	160	120	120	120	120
Taq Polymerase	0.68 U	0.48 U	0.6 U	1.50 U	1.23 U	0.60 U	0.90 U	0.68 U	1.20 U

Table 3-2. Mean heterozygosity (He), probability of identity (pI) and mean number of alleles (A) in wood bison populations. Populations with a '*' are from Wilson and Strobeck (1999a).

	n	He	1/pI	A
HLA	58	0.508	5 600 000	5.55
HLC	33	0.440	410 000	4.82
HLR	26	0.532	38 000 000	5.55
WBNP*	81	0.552	57 000 000	6.55
MBS*	28	0.441	760 000	4.27
EINPW*	36	0.520	1 400 000	3.64

Note: population abbreviations are: captive Hook Lake founders (HLA), calves born to this population (HLC), Hook Lake Region (HLR), Wood Buffalo National Park (WBNP), Mackenzie Bison Sanctuary (MBS), and Elk Island National Park (EINPW).

Table 3-3. Probability of identity values for all loci in each wood bison population. Populations with a '*' are taken from Wilson and Strobeck (1999a). Abbreviations are described in Table 3-2.

	BM143	BM2830	BM4513	BMC1222	BM1225	BOVFSH	Eth121	RT9	RT24	RT27	RT29
HLA	0.185	0.138	1	0.248	0.194	0.054	0.249	0.757	0.360	0.444	0.090
HLC	0.264	0.152	1	0.279	0.236	0.077	0.325	0.882	0.343	0.621	0.197
HLR	0.094	0.082	1	0.291	0.235	0.053	0.204	0.675	0.371	0.346	0.053
WBNP*	0.135	0.119	1	0.212	0.117	0.039	0.202	0.571	0.322	0.488	0.063
MBS*	0.110	0.183	1	0.636	0.269	0.044	0.704	0.423	0.397	0.704	0.103
EINPW*	0.351	0.228	1	0.258	0.229	0.122	0.249	0.354	0.308	0.401	0.114

Table 3-4. Assignment test results. Numbers in brackets are the percent of individuals from the source population assigned to each population. Individuals assigned to their source population are in bold. Population abbreviations are described in Table 3-2.

	Population to which each individual was assigned					
Source	HLA	HLR	WBNP	EINPW	MBS	Plains bison
HLA	**26 (0.45)**	18 (0.31)	7 (0.12)	1 (0.02)	6 (0.10)	0
HLR	12 (0.46)	**5 (0.19)**	6 (0.23)	0	2 (0.08)	1 (0.04)
HLC	25 (0.76)	1 (0.03)	1 (0.03)	1 (0.03)	5 (0.15)	0
WBNP	21 (0.26)	14 (0.17)	**33 (0.41)**	5 (0.06)	5 (0.06)	3 (0.04)
EINPW	2 (0.06)	0	2 (0.06)	**30 (0.83)**	2 (0.06)	0
MBS	3 (0.11)	5 (0.18)	3 (0.11)	0	**17 (0.61)**	0

Table 3-5. D_S (above the diagonal) and D_{LR} (below the diagonal) genetic distance values between all bison populations. D_S values between all plains bison, WBNP, MBS, and EINPW are taken from Wilson and Strobeck (1999a).

	HLA	HLR	WBNP	EINPW	MBS	AISP	CSP	EINPP	FNWR	NBR	PM	WMR	YNP
HLA		0.011	0.018	0.085	0.059	0.203	0.193	0.192	0.254	0.239	0.261	0.242	0.227
HLR	0.05		0.025	0.090	0.043	0.193	0.169	0.185	0.254	0.225	0.247	0.225	0.194
WBNP	0.46	0		0.055	0.074	0.171	0.181	0.155	0.231	0.190	0.210	0.218	0.196
EINPW	2.75	2.93	2.34		0.163	0.280	0.257	0.279	0.377	0.320	0.320	0.303	0.296
MBS	1.82	1.06	1.66	3.96		0.209	0.195	0.193	0.302	0.226	0.251	0.204	0.159
AISP	7.91	7.09	7.07	10.96	8.72		0.261	0.204	0.261	0.215	0.290	0.217	0.184
CSP	5.56	3.93	4.52	8.90	6.47	8.61		0.162	0.151	0.225	0.192	0.172	0.168
EINPP	7.78	6.75	5.44	12.02	7.83	9.16	4.99		0.190	0.174	0.053	0.182	0.126
FNWR	8.14	6.86	7.25	14.40	9.70	12.31	4.64	7.07		0.194	0.272	0.258	0.251
NBR	9.45	7.39	6.22	11.88	8.57	10.26	6.53	5.58	7.32		0.269	0.240	0.230
PM	7.82	6.53	5.33	11.52	7.51	9.68	5.19	0.71	8.76	6.73		0.218	0.141
WMR	8.84	7.35	7.24	11.05	8.93	10.21	5.95	8.29	9.53	7.95	8.33		0.090
YNP	7.65	5.48	5.75	10.13	6.37	8.81	4.98	4.63	8.08	6.60	4.33	4.96	

Note: wood bison populations are abbreviated as: Hook Lake Wood Bison Recovery Project founders (HLA), Hook Lake Region (HLR), Wood Buffalo National Park (WBNP), Elk Island National Park (EINPW), Mackenzie Bison Sanctuary (MBS). Plains bison populations are abbreviated as: Antelope Island State Park (Utah, AISP), Custer State Park (South Dakota, CSP), Elk Island National Park (EINPP), Fort Niobrara National Wildlife Refuge (Nebraska, FNWR), National Bison Range (Montana, NBR), Pink Mountain (British Columbia, PM), Wichita Mountains Wildlife Refuge (Oklahoma, WMR), Yellowstone National Park (Wyoming, YNP).

Table 3-6. Mean and standard deviations for the pairwise relatedness (*R*) values within and among the three Hook Lake Wood Bison Recovery Project cohorts. N is the number of pairwise comparisons.

	Mean *R*	SD	N
1996	-0.072	0.231	153
1997	0.035	0.212	190
1998	-0.028	0.233	190
Among cohorts	-0.020	0.243	1120
Total	-0.020	0.238	1653

Table 3-7. Number of successful reproductive events in 1999 and 2000 by each of the five males born in 1996. None of the younger males were able to successfully reproduce in either year.

	Individual Identification Number				
	31	37	40	44	48
1999	0	5	0	4	0
2000	1	8	0	13	2
Total	1	13	0	17	2

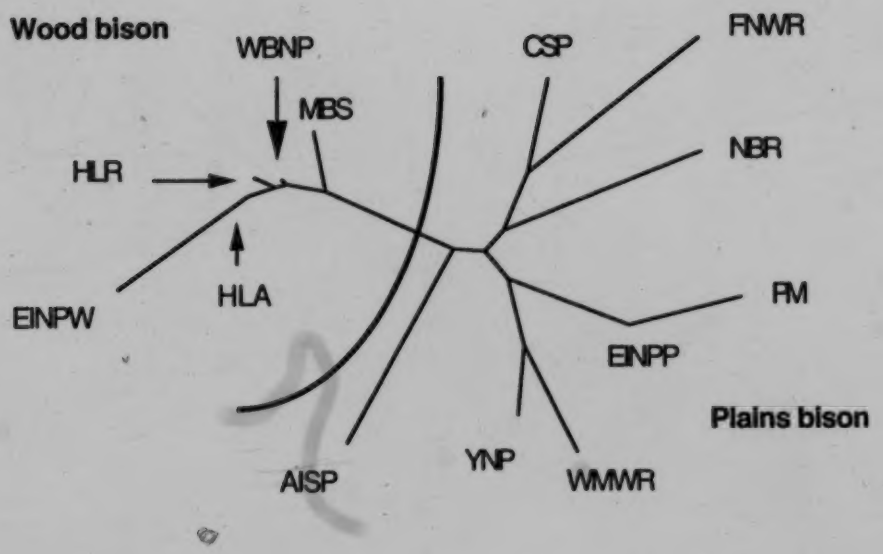

Figure 3-1. Nei's standard unrooted tree designed using the neighbour-joining method. Abbreviations can be found in Table 3-5.

Chapter 4

*Reproductive Success in Wood Bison (*Bison bison athabascae*), a Polygynous Mammal, Established Using Molecular Techniques*[1]

Introduction

Wood bison (*Bison bison athabascae*) were once prevalent throughout northern Canada and Alaska. However, their numbers declined from thousands of animals to a low of about 250 by 1900 (Soper 1941). While wood bison populations have increased in size and numbers since that time, they are currently designated as threatened in Canada and have been extirpated from Alaska (COSEWIC 1998). There are currently three large (more than 300 animals) wood bison populations in Canada, located in Wood Buffalo National Park (Alberta and Northwest Territories), Mackenzie Bison Sanctuary (Northwest Territories), and Elk Island National Park (Alberta). The Mackenzie Bison Sanctuary and Elk Island National Park populations were established from Wood Buffalo National Park with a small number of animals (less than 20) in the 1960s. Unfortunately, the Wood Buffalo National Park population, while the most genetically variable (Wilson and Strobeck 1999a), is infected with tuberculosis and brucellosis. Therefore, the brucellosis- and tuberculosis-free Elk Island National Park population has been used as a source for the establishment of a number of new wood bison populations. Elk Island National Park is a semi-wild population, in that it is fenced and annual reductions occur.

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[1] A version of this chapter has been submitted for publication. Wilson, Olson & Strobeck (submitted) *Can. J. Zool.*

The reproductive success of wood bison is largely unknown. The number of potentially reproductively successful males and females in a population, and the causes of this success, would be useful information in determining how many individuals to found a herd with, and the growth expected in this herd. As newly founded populations could be negatively affected by inbreeding depression, it would also be beneficial to know if bison practiced inbreeding avoidance. Trivers and Willard (1973) raised the possibility that, in polygynous species where males are more costly to raise than females, less fit cows should preferentially produce female calves. Some have suggested that cows that calved the previous year are in poorer condition than barren cows (Green and Rothstein 1991). This could affect the sex ratio in populations founded in suboptimal areas. In order to address these issues, we attempted to examine a number of different aspects of reproduction in bison. First, what proportion of males are reproductively successful in a breeding season, and what factors affect this success? Secondly, how many females produce calves in a season, and can a predictive model be established for female reproductive success? Do cows that are in poor body condition due to calf production produce fewer male calves? And lastly, are breeding pairs less closely related than expected, i.e. are bison practicing inbreeding avoidance?

Mating Behaviour in Bison Males

The number of reproductively successful males in a population will be largely governed by their breeding system and mating behaviour. Like most large mammals, bison have a polygynous mating system. They are highly sexually dimorphic, with males being on average 1.6 to 1.8 times the size of females (Olson 2001). Most mating occurs during the rut, which lasts four to five weeks (Meagher 1973, Lott 1981, Melton et al. 1989). During this time, males join the large cow herds and compete for females. They tend a

single female until she is ready to copulate, and then leave after guarding her for a short period of time, presumably to protect against sperm competition (Lott 1981). Males may be challenged by a large number of competitors between the time they start to tend a female and the time copulation occurs, and tending males are often replaced (Lott 1979). Males usually leave the cow herd for a time during the rut, presumably to recuperate, and may return to it later (Komers et al. 1992, Wolff 1998). The absence of these bulls may allow lower ranked males to breed. Male breeding success is strongly correlated with success in aggression with other males (Lott 1979). However, male rank changes often during this period, as they tire from aggressive interactions (Lott 1981, Rutberg 1986a). In fact, Lott (1979) found that most dyads had dominant-subordinate role reversals at least once during a three-week study period. Between 50 and 73% of males have been observed to mate successfully each year (Lott 1981, Wolff 1998).

The ability of an individual bull to successfully compete for mates may depend on a number of factors, such as age and weight. Males are able to produce viable sperm as yearlings (Haugen 1974), and two-year-olds are capable of successfully reproducing when older males are absent from the population (see Chapter 3, this thesis). However, prior studies have shown that they do not participate in the rut until the ages of five or six, when they are large enough to achieve high status (Lott 1981, Maher and Byers 1987). There are a number of reasons younger bulls may not be successful during the rut. For instance, they may not be as fertile as older ones, and do not have as much subcutaneous fat to use as energy reserves during aggressive interactions (Komers et al. 1994b). Older bulls seem to increase their reproductive effort more than younger ones during the rut, and win more aggressive interactions (Maher and Byers 1987, Komers et al. 1992, Komers et al. 1994c). However, some studies have found that social standing (or fighting ability) does not correlate with age or weight (Lott 1979, Wolff 1998). Instead, males may have a certain breeding potential, which they maintain for most of their life. If this

is the case, then prior reproductive success may be predictive of current reproductive success.

The subspecific designation for wood bison is a contentious issue (see for e.g. Geist 1991, Van Zyll de Jong et al. 1995, Polziehn et al. 1996). For the purposes of this study, we will continue to use the subspecific designation for wood bison, while allowing for the possibility that "race" may be a better classification. To date, most studies of bison mating behaviour, including those outlined above, have been obtained from plains bison (*Bison bison bison*). There is some evidence that wood bison males may behave differently than plains bison during the rut. Wood bison herds decline in size during the rut, as opposed to the large aggregates seen in plains bison (Soper 1941, Melton et al. 1989). Whether there is a genetic basis for these differences in group size between the subspecies, or it is just a response to different habitats, is unclear (Melton et al. 1989). The smaller size of these herds may make them more controllable by a few dominant males, resulting in a different mating system between wood and plains bison (Calef and Van Camp 1987). Wood bison seem to be more solitary than plains bison during the rut, with most aggressive interactions occurring when a lone male clashes with the dominant male in the cow herd he is trying to join (Melton et al. 1989). Wood bison may then have a harem formation system, as opposed to the dominance hierarchy seen in plains bison. If this is true, a smaller proportion of wood bison than plains bison should be reproductively successful each year. It should be noted, however, that the dominant males within harems do change throughout the breeding season (Komers et al. 1992).

Fecundity in Bison Females

Bison cows usually produce their first calf at three, although some may calve at two (Fuller 1961, Haugen 1974, Meagher 1973, Reynolds et al. 1982, Shaw and Carter 1989).

Calving rates can range from 35 to 100%, depending on the population (McHugh 1958, Meagher 1973, Lott 1979, Lott and Galland 1987, Kirkpatrick et al. 1993). Mackenzie Bison Sanctuary, the only wood bison population for which this data is published, had a cow:calf ratio of 0.38 (Komers et al. 1994c).

A number of factors have been examined to determine their relationship with an individual cow's fecundity. Some have suggested that bison follow a two- or three- year calving pattern due to the nutritional cost of producing calves (Soper 1941, Meagher 1973, Kirkpatrick et al. 1993). Conversely, Komers et al. (1994a) found that lactating females were more likely to ovulate than non-lactating females. Others have found no evidence for a pattern in how often females calve (Lott and Galland 1985b, Shaw and Carter 1989, Green and Rothstein 1991). While age has been predictive of reproductive success, no relationships have been found between either fecundity or dominance (and access to resources) and weight (Rutberg 1986a, Green and Rothstein 1991). Fecundity and dominance should be related in populations where access to resources is limited, if greater access to resources increases the body condition of a female.

There may be a relationship between cow body condition and calf gender. Trivers and Willard (1973) argued that in polygynous species where variance in male reproductive success is large, cows in good body condition should produce more male offspring than those in poorer condition. Females that have not had the demands of raising an offspring in one year may be in better physical condition than those that have, and may therefore produce more male calves (Trivers and Willard 1973, Clutton-Brock and Albon 1982). Bison cows have been found to weigh more when barren than after calving, suggesting that the former are in better condition (Green and Rothstein 1991). Also, raising a male calf is more costly than raising a female calf due to the size dimorphism in this species. This may reduce the body condition of cows, and therefore their fecundity, the year following the birth of a male calf (Wolff 1988). In some

populations, female bison that were not lactating produced more male offspring than those that were (Rutberg 1986b). This suggests that non-lactating females may in fact be in poorer body condition than females that have reproduced. Other populations have shown no pattern between calf gender and fecundity in either the previous (Shaw and Carter 1989), or following (Wolff 1998) year. We tested the Trivers-Willard hypothesis in the Elk Island National Park population by examining the correlation between calf gender and fecundity from year to year.

Inbreeding and Inbreeding Avoidance

Many studies have illustrated the deleterious effects of inbreeding in populations (Pusey and Wolf 1996). Individuals that have closely related parents will have a lower heterozygosity. One of the costs of inbreeding is supposed to be lower fitness. Therefore, individuals with low heterozygosity should have lower reproductive success. If calf parentage is known, then an individual's reproductive success can be compared with its observed heterozygosity to determine if there is a relationship between these two variables. Extreme cases of inbreeding may lead to extinction of local populations (Saccheri et al. 1998). It is therefore possible that natural selection has resulted in a mating system where breeding with close relatives is avoided (Pusey and Wolf 1996). The genetic relationships between mated pairs can be examined and compared with the rest of the population to determine if bison select mates that are less related than expected by chance.

Objectives and Overview

The four main goals of this study were to: i) to determine the levels of differential reproductive success in bison, ii) design a predictive model of reproductive success, taking into account factors such as age, weight, and prior success, iii) evaluate the Trivers-Willard hypothesis with regards to the gender of calves produced by barren and non-barren females, and iv) examine the amount of inbreeding that occurs in bison populations, and the potential negative effects of this inbreeding on an individual's reproductive success. In order to address these issues, we performed a parentage study on the calves born to Elk Island National Park between 1996 and 1999. Elk Island National Park is an ideal location for this study because over 90% of the population is handled every year, making sample collection from calves and potential parents relatively straightforward. Animals are also weighed when handled, and calves given an eartag corresponding to their year of birth.

The reproductive potential of an individual is better measured by an evaluation of its actual fecundity, rather than mating success. In many instances, observed mating success is not predictive of reproductive success (Hughes 1998). This is especially true for species where there are frequent changes in the dominance hierarchies (see for e.g. Coltman et al. 1999). Bison fall into this category, as dominant-subordinate relationships between males usually change at least once per breeding period (Lott 1979). Therefore, genetic techniques may be preferable for establishing paternity in some species. For example, genetic techniques revealed that many songbirds, once thought to be monogamous, participate in some degree of extra-pair matings (Birkhead et al. 1990, Petrie and Kempenaers 1998). Also, genetic studies in some seal species have shown that male mating behaviour may not be a reliable indicator of reproductive success (Harris et al. 1991, Worthington Wilmer et al. 1999). There are also a number of reasons to suspect

that observed mating success may not be indicative of reproductive success in bison. Generally, cows are tended before mating occurs. They can be tended by up to ten males and are sometimes seen to breed with more than one bull, making determination of parentage through mating observation difficult (Lott 1981, Wolff 1988). Tending, and even copulation, may not be indicative of estrous - and therefore the potential for conception - in bison cows (Lott 1981, Komers et al. 1994a). Mating behaviour of all bison may not be easily observable. Mating may occur at night (Lott 1979, Wolff 1998), and copulation events are rarely observed in populations where animals can hide in the vegetation (Komers et al. 1994a). The observation of a cow with a calf also may not be indicative of reproductive success for females, as calf mix-ups may occur in social species with precocious young, like bison (Lott 1973, Clutton-Brock and Guiness 1975). Such mix-ups are thought to be unlikely in bison, although possible if parturition occurs within the herd (Lott and Galland 1985a). Therefore, genetic techniques may be a better measure of reproductive success than observations of mating behaviour in wood bison. The determination of parentage in my study was performed using a suite of 21 microsatellite loci. DNA microsatellites have already proven useful in resolving paternity of bison calves (Mommens et al. 1998, Schnabel et al. 2000).

Materials and Methods

Sample Collection

The Elk Island National Park wood bison population was established in 1965 with 21 animals from Wood Buffalo National Park. However, brucellosis was detected in these bison, so the original animals were destroyed and their eleven calves used as the founders

for this population. Elk Island National Park is fenced, and the largest predator in the park is the coyote. Coyotes (*Canis latrans*) have been documented to hunt bison calves (S. Pruss pers. comm.), but their effect on the population is thought to be negligible. Therefore, annual reductions of the population must take place. About 90% of the wood bison are rounded up and handled in January of each year. During the annual roundups, bison are vaccinated against several bovine diseases and weighed. Unique eartags designating the year of birth are also given to the calves, and any animal that lost its original eartag. If these animals were sampled in prior years, their genotypes could be matched up. Otherwise, age data was unavailable. Therefore, age and weight are available for a large number of the bison in this population. The wood bison population is managed at a size of about 350, after the annual reduction of around 60 animals. During the roundups from 1997-2001, hair samples were taken from each handled animal, and the eartag of that animal recorded. Only the calves born in 1996-1999 were included in this study, but samples from animals in the 2001 roundup that had not previously been seen were also analyzed. Samples consisted of at least 20 hairs with roots, and were stored in envelopes at room temperature.

Laboratory Methods

DNA was isolated from 10 to 20 roots of the 613 sampled animals using a QIAamp® Tissue Extraction Kit. The following microsatellite loci were used in this study: BOVFSH (Moore et al. 1992), BM1225, BM4440, BM723, BM757, BM5004, BM3507, BM1824, BM2830, and BMC1222 (Bishop et al. 1994), RT29 (Wilson and Strobeck 1999a), TGLA57 (Barendse et al. 1993), CSSM022 (Barendse et al. 1994), Eth152 (Steffen et al. 1993), NVHRT30 (Roed and Midthjell 1998), TGLA126, TGLA261, TGLA53, AGLA269, and AGLA232 (Georges and Massey 1993), and BBJ24 (Wilson

and Strobeck 1999b). FAM, HEX, or TET fluorescent labels were added to one primer from each of these sets. TGLA53, TGLA57, TGLA126, CSSM022 and BM1824 had their unlabelled primers tailed to promote adenylation of the PCR product (Brownstein et al. 1996). When possible, loci were multiplexed (amplified in the same reaction during PCR). Each PCR reaction contained 2.5 mM $MgCl_2$ and PCR buffer (10 mM Tris buffer, pH 8.8, 0.1% Triton X100, 0.16 mg/mL BSA, 50 mM KCl). Multiplexes and the remainder of the PCR contents can be found in Table 4-1. *Taq* polymerase was purified as described in Engelke et al. (1990). PCR cycling conditions were as follows: 1 min at 94°C; 3 cycles of 30 s at 94°C, 20 s at 54°C, 5 s at 72°C; 33 cycles of 15 s at 94°C, 20 s at 54°C, 1 s at 72°C; 30 min extension at 72°C. PCR reactions were performed on an ABI 9600 thermal cycler and electrophoresed on an ABI 373A or ABI 377 DNA Sequencer.

Data Analysis

All loci examined were tested for heterozygote deficiency with GENEPOP v3.1d, which uses a Markov chain algorithm (Raymond and Rouset 1995). Linkage disequilibrium between loci was also examined using GENEPOP v3.1d software. The genetic variation at each locus was calculated using three different methods: number of alleles, average level of parent-offspring exclusion (Paetkau and Strobeck 1997), and paternal exclusion given the mother's genotype (Chakravarti and Li 1983). Calves were excluded from each of these analyses.

The parents of all 317 of the calves born during the study period were unknown. Parentage of the calves was calculated with three different methods. Exclusion was used, where parentage was assigned to the only male and female (or male-female combination) that matched the calf's alleles at all loci. Parentage was also determined with CERVUS

1.0 (Marshall et al. 1998), which uses a likelihood method. Unlike exclusion, CERVUS 1.0 allows for the possibility of a mismatch between the parent and offspring genotypes as a result of mutation or human error. It is also fairly robust to the confounding effects of potential parents being related to one another. Slate et al. (2000) have demonstrated the ability of CERVUS 1.0 to establish parentage in large, natural populations. CERVUS 1.0 was used to determine parentage in the Elk Island National Park population using two different stepwise manners. First, maternity was assigned to any female who matched the calf at the 95% confidence level. These maternity assignments were then considered to be known, and used to determine the paternity of the calf. In the second method, we attempted to assign paternity to the calves first, and then used the presumed fathers to assign maternity. The CERVUS 1.0 simulations required for parentage assignment were performed with the following parameters: for males, 10000 cycles, 150 potential parents, 90% of candidate parents sampled, 99% of loci typed, and 1% of loci mistyped. Parameters for the females were: 10000 cycles, 165 potential parents, 95% of candidate parents sampled, 99% of loci typed, and 1% of loci mistyped. The typing error rate was an overestimate, based on the number of mistakes found after retyping approximately 30% of the population. We only considered a CERVUS 1.0 result a true assignment of parentage if it had a confidence level over 95%.

The number of agreements and disagreements in parentage assignment using these three methods can be found in Table 4-2. Results from the methods of determining parentage were amalgamated, and the following criteria were used to determine whether the parentage assignment would be accepted. Parentage assignments were accepted if: 1) the three methods assigned the same parent, 2) two methods assigned the same parent, the other assigned no parent, and the parent (or parents, if both assignments were possible) mismatched the calf at one or fewer loci, 3) one method assigned a parent, the other two did not, and that individual plus the other assigned parent mismatched the calf at one or

fewer loci, and 4) two methods disagreed, but one proposed set of parents mismatched the calf at one or fewer loci and the other did not. No other parentage assignments were accepted.

In order to determine the effect of age, weight, prior success and heterozygosity on reproductive success in males and females, correlations and a linear regression were performed using SPSS v6.1. Three "dummy", or "indicator" variables were also included in the regression to allow for differences in each of the study years. Missing data in the linear regression were treated by pairwise exclusion. Variables were added to the linear regression model using the "forward" option, where variables are entered into the formula one at a time. The r^2 value for the new formula is tested against that from the old formula to determine if the addition of the new variable makes a significant change in the ability of the model to predict the value of the independent variable. We used a p-value of 0.05 as the cut-off for the addition of new variables into the models. In males, reproductive success was defined as the percentage of calves an individual sired each year, and prior success was the percent sired in the previous year. In females, reproductive success was considered the number of calves produced each year, and prior success was the number produced in the previous year.

To determine if inbreeding avoidance was occurring in the Elk Island National Park wood bison population, pairwise *R*, or relatedness coefficient, values were generated from the allele frequencies of the adults in this population using RELATEDNESS 5.0.6 (Goodnight and Queller 2000). In each of the four years, pairwise *R* values between a calf's parents were compared to *R* values between pairs of individuals that did not mate. If inbreeding avoidance was occurring, the mean *R* values between mated pairs should be lower than that seen between unmated pairs.

Results

None of the 21 loci were deficient in heterozygotes in the Elk Island National Park population ($p>0.05$). Of the 210 locus pairwise tests for linkage disequilibrium, 13 were found to be significant when the Dunn-Sidak experiment-wise error rate was used ($p<0.05$, Sokal and Rolf 1995). Of the significant comparisons, AGLA269-BM1824, BM1824-TGLA261, and TGLA126-TGLA57 are known to be on different chromosomes in cattle (Barendse et al. 1994, Bishop et al. 1994). BM757 and TGLA261 are on the same chromosome, but the distance between them (almost 50 cM) should make them appear unlinked (Bishop et al. 1994). BM2830 and Eth152 are approximately 10 cM apart in cattle (Bishop et al. 1994), but we decided to use both of these loci in the analysis due to their relatively high levels of genetic variation. Also, BM2830-BOVFSH and BMC1222-BOVFSH, which were in linkage disequilibrium in this study, were not found to be in linkage disequilibrium in a prior study of bison populations (Wilson and Strobeck 1999a).

The genetic variation present at each of the surveyed loci in this population can be found in Table 4-3. The wood bison population at Elk Island National Park is one of the least variable bison populations (Wilson and Strobeck 1999a). Therefore, it was necessary to use a fairly large number of loci in order to get the levels of exclusion we desired.

We assigned maternity and paternity to 295 and 253 of the 317 calves, respectively (Table 4-4). In each year, more maternities were assigned than paternities. The number of paternities assigned increased from about 60% in 1996 to over 90% in 1999. The number of assigned maternities was more constant, ranging from 88% in 1996 to 97% in 1999.

The number of reproductively successful males and females each year of the study can be found in Table 4-5. While the number of sampled reproductively successful males increased over the study period, the proportion of successful males stayed relatively constant at 35-40% (Table 4-5A), suggesting that the males which could not be rounded up in the first years of the study were not more successful than those that were. Sixty-one percent of the sampled mature males were reproductively successful at some point in the study period. This value is an underestimate as males that reached maturity during the later years of the study, and therefore could not have mated in the earlier years, were included. If age data for a male became unavailable due to the loss of an eartag, maturity was estimated as the time the animal reached a weight of 675 kg.

In contrast to the relatively constant proportion of successful males, proportion of successful females ranged from 50-70% (Table 4-5B). Eighty-one percent of the sampled females were reproductively successful in at least one of the years of this study. Again this is an underestimate, due to the inclusion of females that were not mature over all four years.

The age structure of reproductively successful and unsuccessful males in each of the study years are displayed in Figure 4-1A-D. The proportion of calves produced per year by males in each age class is summarized in Table 4-6. Only males of known age are included. Note that 'age' was calculated as the age of the male during the rut previous to the year the calf was born, i.e. when the calf was conceived. The number of known-aged animals increased over the study period, as the genotypes of animals that were retagged in later years could be compared with all other individuals in the database. In this way, many retagged animals could be matched up with their original genotype. The age of reproductively successful males ranged from five to fourteen. Two five-year-olds were successful: one in 1998 and one in 1999. Two fourteen-year-olds were successful in the same years. Most of the successful males were in the seven to ten age classes. How

well the age structure in this population represents the age structure in natural populations is unknown.

Age structure of reproductively successful and unsuccessful females can be viewed in Figure 4-2A-D, and age structure of successful females is summarized in Table 4-7. The age structure of successful females is not as sharply peaked as that of the males. 'Age' was calculated as the age of the cow during the rut prior to the birth of her calf, i.e. when the calf was conceived. The age of reproductively successful females ranged from two to twenty. Two 2-year-olds were bred: one in 1996 and one in 1999. A single 20-year-old reproduced in 1999. Most individuals aged five to fourteen were able to reproduce.

The age at first reproduction of the males and females that reached breeding age at the start of the study period are presented in Tables 4-8A and B, respectively. Mean age of first reproduction for males was 7.7 (variance 1.0), and 3.7 (variance 1.2) for females. For this calculation, individuals that did not breed during the study period were assumed to breed in the next year. Males born in 1991 were not included in this analysis as the sample size was two, and neither were successful in any year.

The average pairwise *R* values for reproductive pairs and individuals that did not mate for each of the years in the study period can be found in Table 4-9. There were no significant differences between these values in any of the years (t-test, $p>0.05$)

Spearman's correlation coefficient was used (SPSS v6.1) to examine the relationship among individual reproductive successes in each of the years for both males and females. For males, where reproductive success was defined as the proportion of calves born in a specific year sired by each individual, the relationships between reproductive success in 1996-1997 ($r=0.45$, $p=0.00$), 1997-1999 ($r=0.27$, $p=0.02$), and 1998-1999 ($r=0.57$, $p=0.00$) were all significant. In females, where reproductive success was defined as the absolute number of calves produced each year, the only significant

relationships were between the years 1996-1998 (0.20, p=0.05), and 1997-1999 (0.31, p=0.00).

In males, correlations were significant between reproductive success and age (r=0.16, p=0.02), success and prior success (r=0.41, p=0.00), success and weight (r=0.30, p=0.00), prior success and age (r=0.21, p=0.02), prior success and weight (r=0.25, p=0.00), and age and weight (r=0.33, p=0.00). The prior success and weight variables led to a significant change in r^2 for the linear regression model. Male reproductive success was best described using the formula (success=-2.49 + 0.357(prior success) 0.00077(weight), where weight is measured in kg, r^2=0.21).

Female reproductive success was significantly correlated with weight (0.50, p=0.00), and each of the dummy variables used to represent the year the calf was born in. Age and heterozygosity (r=0.111, p=0.01), age and weight (r=0.53, p=0.00), prior success and weight (0.164, p=0.00) and weight and heterozygosity (r=0.107, p=0.01) were also correlated. Weight, age, the dummy variable for calves born in 1997, and prior success were included in the final linear regression model. The formula to best describe female reproductive success was (success=-1.42 - 0.027(age) + 0.00092(weight) + 0.14(1997) - 0.11(prior success), where age is in years old in the rut previous to the calving season, weight is in kg, and 1997 has a value of '1' for the 1997 calving season, and '0' otherwise, r^2=0.33).

Reproductive success for all males and females who were mature and present in the population during all four years of the study are in Figures 4-3A and 4-3B, respectively. The most successful males seemed to be between the ages of eight and twelve, although 14 year olds were also quite successful. During this period, males fathered between zero and 24 calves, with a mean of 3.5 (variance 14.9). Female reproductive success ranged from zero to four, with a mean of 2.7 (variance 1.0).

Eighteen females followed a two-year reproductive pattern, while 34 females reproduced in three of the four years, and could be following a three-year reproductive pattern.

Chi-squared tests for independence were performed to examine the relationship between the gender of the calf born to a female the previous year, with her reproductive success in the current year for the 1996-1997, 1997-1998, and 1998-1999 periods. Females were classified as being barren, having a male calf, or having a female calf in each year. Only in the 1996-1997 period was the relationship between calf gender and reproductive success significant ($p<0.05$). Cows barren in 1996 were more likely to have daughters and less likely to have sons in 1997 than expected.

Discussion

Our system of establishing parentage with the use of a suite of 21 microsatellites identified paternity in 80% and maternity in 93% of the sampled calves from Elk Island National Park, which is among the least variable bison populations (Wilson and Strobeck 1999a). This suggests that this system would be useful in situations where parentage must be established without prior knowledge of potential parents. The use of both exclusion and CERVUS 1.0 enabled us to establish more parentage assignments than if we had used only one of these systems alone.

Thirteen of the pairs of loci were in linkage disequilibrium. Loci found to be in linkage disequilibrium are not necessarily linked. Linkage disequilibrium may also occur if populations have recently increased or decreased in size, or inbreeding is prevalent (Weir 1996). The founding of Elk Island National Park from only 11 individuals about 35 years ago and subsequent increase in size to about 350 is likely the reason for the linkage disequilibrium seen in this population. However, the possibility cannot be ruled

out that some of the loci for which map data are not available are closely linked. While using loci that are linked may decrease our system's power in establishing parentage, it will not increase the number of misassignments.

The number of paternities assigned increased from about 60% in 1996 to over 90% in 1999. This was likely due to the increase in number of males genotyped over the course of this study. A greater number of males that were reproductively successful in 1996 were likely not rounded up, or died before they could be sampled. This trend was also evident to a lesser extent in the maternity assignments.

Male Reproductive Success

Male differential reproductive success was evident in this population, with males producing between zero and 24 calves over the length of this study. The youngest male was competitive in the rut by five years of age, and the oldest was 14. However, reproductive success seemed to peak in the seven to ten range. The average age of first reproduction for males in this study was 7.7. Despite the fact that younger males are able to enter the rut when older males are excluded (Chapter 3, this thesis), they likely do not have the energy resources to tend females very long (Komers et al. 1994b) and hence were not able to achieve high levels of reproduction. Similar age structures of successful males and ranges in number of calves sired were found in plains bison populations (Maher and Byers 1987, Wolff 1998).

About 40% of mature males were reproductively successful each year, which is lower than that seen in plains bison populations (50-73%, Lott 1981, Wolff 1998). This could be due to potential differences in the mating systems of wood and plains bison. In contrast to the large plains bison groups, wood bison may form small harems during the rut, which they defend against visitors (Soper 1941, Melton et al. 1989). The smaller size

of these harems may make them easier to defend than larger groups, allowing fewer males to dominate breeding. However, there is still extensive interchange of dominant males within wood bison herds, as they leave for periods of time to recuperate (Komers et al. 1992). Another possibility is that the lower proportion of reproductive males could be due to differences in the age structures of these populations. While some younger males were able to breed in the Elk Island National Park population, most of the mating was limited to males seven or older. Differences in proportion of successful males could be explained if the Elk Island National Park population contained more of these younger males than the other studied populations.

A linear regression model showed that prior reproductive success and male weight could be used to predict reproductive success. However, the ability to explain variation in success was fairly weak (r^2=0.21). Other studies have found no significant relationship between weight and reproductive success (Lott 1979, Wolff 1998). It should be noted that our weight data was collected in January, about four months after the end of the rut, and therefore may not be a reliable estimate of pre-rut weight. Komers et al. (1994b) found that bulls aged six to ten lost most of their subcutaneous fat during the rut, while younger bulls did not. However, by the time weight data was collected, the males were likely able to regain some of the weight lost during the rut.

Adding age and sampling year did not increase the ability of the model to estimate reproductive success. Relationships between age and activities related with mating, such as fighting and tending, have been found in studies of wood and plains bison (Komers et al. 1992, 1994c, Maher and Byers 1987). However, others have found no such association (Lott 1979, Wolff 1998). Older males in Elk Island National Park may be increasing attempts to gain matings, but this is not reflected in an increase in reproductive success.

The ability to make a good predictive model of reproductive success could be hampered by the fact that small differences in individual fitness may allow other bulls to become dominant after minor changes in physical prowess (Lott 1979). Also, males of lower social status may reduce competition by rutting at different times (Komers et al. 1994c). Other factors such as testosterone levels, or overall health, could play a role in male reproductive success. Bison bulls may achieve a certain level of breeding potential and maintain it for most of their lives (Lott 1979). This is supported by the positive correlations between breeding success over three of the years in our study, and the ability of prior success to increase the predictiveness of the linear regression model. Also, since including variables to signify the year the calf was conceived did not significantly increase the predictiveness of our model, there was no apparent relationship between breeding success and annual environmental differences. Males seemed to have the about the same reproductive potential over our study period, regardless of age and only slightly dependent on weight.

Female Reproductive Success

The age of reproductively successful females in our study ranged from two to 20. Females were successful over a longer period of years than males, and their reproductive success was not as sharply peaked. Peak reproductive age was between five and 14. Note that 'age' was calculated as the age of the cow during the rut prior to the birth of a calf i.e. at the time of conception. Only two 2-year-olds successfully produced offspring over the course of this study, and the mean age at first reproduction for females was 3.7. This is in contrast to most other studies, which suggest that females primarily start participating in the rut at two (McHugh 1958, Fuller 1961, Green and Rothstein 1991).

Therefore, younger females in Elk Island National Park were not as fecund as those in other populations.

Reproductive success varies between populations, and is influenced by such factors as climate (Verme and Doepker 1988), and nutrition (Verme 1969, White et al. 1989). Bison herds existing in harsher environments have lower reproductive success (Lott and Galland 1985b). Each year, 68-84% of females at Elk Island National Park produced calves during the length of this study. The lower range of values was consistent with bison populations that suffer from poor nutrition, or live in harsher environments (Fuller 1962, Lott and Galland 1987, Komer et al. 1994c, Kirkpatrick et al. 1996), and was less than that seen for bison living in locations where nutrition and environment have less effect on reproduction (Haugen 1974, Lott 1979, Rutberg 1986, Shaw and Carter 1989). The wood bison at Elk Island National Park compete for food with hundreds of wapiti (*Cervus elaphus*) in the grasslands and sedge meadows (W. Olson, pers. comm.). This could have resulted in the relatively low level of reproductive success and high age at first reproduction seen in this study. Forage in Elk Island National Park has been increasing in recent years, while the numbers of wapiti and bison are not allowed to increase. If wood bison reproduction is negatively affected by the amount of competition for food with wapiti, the increase in available forage should decrease the age at first reproduction, and increase reproductive success, in this population.

Differential female reproductive success was also evident in this population. Female success ranged from zero to four, the maximum since twinning in bison is rare (McHugh 1958, Fuller 1961),with a mean of 2.7. There was no correlation in fecundity from year to year, suggesting that Elk Island National Park bison did not follow a strict two- or three- year calving cycle, unlike that seen by Kirkpatrick et al. (1993). Others have also found no evidence for a calving pattern in bison (Lott and Galland 1985b, Shaw and Carter 1989, Green and Rothstein 1991). However, there was a positive correlation

between current reproductive success and reproductive success two years prior. It is possible that some cows followed a two-year calving cycle, resulting in the positive correlations two years apart, but the negative correlation between successive years expected for these individuals was lost due to the number of females in the population who did not follow this cycle. The linear regression model showed that lactating females were less likely to produce calves the following year. It should be noted, however, that this was the variable that had the weakest effect on predicting reproductive success that was still included in the model. The nutritional cost of raising calves at Elk Island National Park negatively affected the chance of producing offspring the following year.

Fecundity in females has been found to increase with body weight in many species (see for e.g. Mitchell et al. 1976, Berger 1986). However, Green and Rothstein (1991) and Rutberg (1986a) found little correlation between weight and ability to produce calves in bison. We found a significant correlation between weight and reproductive success at Elk Island National Park. Likewise, multiple linear regression showed that reproductive success is dependent on weight, age, year, and prior success. This suggests that heavier females were more fit, and therefore more able to produce offspring. However, it should be noted that our weight data was collected in January, and may therefore be partially confounded with the weight of the fetus.

Prior studies have found associations between age and reproductive success or dominance in bison cows (Rutberg 1986a, Kirkpatrick et al. 1996). Dominance could increase reproductive success if food is limited. Age also significantly enhanced our linear regression model. However, the relationship between age and reproductive success was negative. Older cows in the population were not as fecund as younger ones during this study. Environmental effects also have a role in determining a female's reproductive success, as evidenced by the inclusion of one of the sampling year dummy variables in the linear regression model. This is expected as differences in population reproductive

success in various bison herds have been attributed to environmental factors (see discussion above). Environmental effects which may play a role in establishing reproductive success each year could include any environmental or habitat factors which may vary between years. For example, forage quantity and quality likely varied during this study, depending on such factors as the amount of rainfall, sunlight hours, etc. An increase in forage should also increase the condition of individuals in the population, and hence reproductive success that year.

The Trivers-Willard (1973) hypothesis that cows in good condition should produce more male offspring was not supported in our study. Only once was the production of a male, female, or no calf in one year significantly dependent on what the cow produced the previous year, and that was apparently due to cows barren the previous year producing less males and more females than expected. If barren cows are more fit than lactating ones (Green and Rothstein 1991), the Trivers-Willard hypothesis suggests that they should be more likely to produce male offspring (Rutberg 1986b). As this was not the case, it is possible that barren cows are less fit, and did not have a calf the previous year due to poorer physical condition. The greater cost of raising a male calf did not decrease fitness to the point where reproductive success in the next year was affected (Wolff 1998).

Inbreeding Avoidance

Mean pairwise relatedness (*R*) values were no different between mated pairs and non-mated pairs in any of the years. Mating essentially occurred at random between individuals that were not more or less related to one another than expected by chance. Bison were not actively avoiding mating with others that were closely related. Instead, they likely avoid close inbreeding through their social system. Bison travel in large, fluid

herds that interchange frequently (as much as once a day, Lott and Minta 1983, Komers et al. 1992). For the most part, males exist outside these herds and do not develop any lasting or consistent relationships with cows (Lott 1979). Most herds are likely collections of unrelated individuals, and bulls choose one or more of these groups to enter during the rut. In this way, the chance of a bull encountering a closely related cow, and successfully mating with it, are low. Since heterozygosity was not correlated with mating success in either males or females, there does not seem to be a large cost for inbreeding in this population. However, it is possible that highly inbred individuals do not survive to adulthood, and therefore were not included as potential parents in this study.

Summary

Male reproductive success was harder to predict than female reproductive success in bison at Elk Island National Park. Male weight and prior success were the variables that predicted reproductive success, and explained 21% of the variance. In contrast, a linear regression model including weight, age, dummy variables for the year of sampling, and prior success explained one third of the variance in female reproductive success. Age and year sampled do not have a predictable effect on male reproduction. Animals from Elk Island National Park are used in the effort to conserve wood bison by establishing more populations in northern Canada. To maximize reproductive potential of these new populations, one may wish to use larger males between the ages of seven and ten, and larger females between the ages of four and five. However, without knowing whether the males have a history of being reproductively successful, there is no guarantee that they will be able to breed with a number of cows. Also, as the bison will be taken into a new location, environmental differences could effect the reproductive success of the cows. Of equal importance is the fact that younger males are capable of reproduction in the

absence of older competitors. While this study lays the groundwork for establishing the factors that effect reproductive success in wood bison through the use of genetic techniques, much of the variance between individuals is still unexplainable.

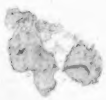

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Table 4-1. Ingredients in the PCR reactions. *Taq* polymerase is expressed in units, while all other measurements are in μM.

	1	2	3	4	5	6	7	8	9	10
Each Primer A	0.19	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Each Primer B	0.19	0.17	0.16	0.17	0.17	-	-	-	-	-
Each Primer C	0.16	0.19	-	-	-	-	-	-	-	-
dNTPs	160	160	160	160	160	120	120	120	120	120
Taq Polymerase	1.24	1.51	1.06	1.21	1.51	0.60	0.90	1.00	1.11	0.80

Note: loci are amplified in the following reaction mixes: BM5004 (locus 1A), BBJ24 (1B), TGLA57 (1C), BM4440 (2A), BM723 (2B), BM1824 (2C), BM2830 (3A), BM1225 (3B), TGLA261 (4A), AGLA269 (4B), BM3507 (5A), BMC1222 (5B), Eth152 (6), CSSM022 (6), RT29 (6), AGLA232 (6), NVHRT30 (6), BOVFSH (7), TGLA126 (8), BM757 (9), and TGLA53 (10).

Table 4-2. The number of agreements and disagreements of parentage assignment using exclusion (exc), CERVUS 1.0 by assigning mother, and then father in a stepwise manner (m-d), and CERVUS 1.0 by assigning father and mother in a stepwise manner (d-m). Numbers in brackets are the actual number of parentage assignments accepted.

	1996		1997		1998		1999	
	Maternity	Paternity	Maternity	Paternity	Maternity	Paternity	Maternity	Paternity
All agree[1]	61 (56)	52 (37)	48 (46)	42 (36)	44 (43)	49 (47)	60 (60)	56 (53)
exc, d-m agree[2]	7 (7)	1 (1)	6 (6)		14 (14)	1 (1)	13 (13)	2 (2)
exc, m-d agree[2]		11 (11)		10 (10)		8 (8)		11 (11)
d-m, m-d agree[2]	5 (5)	7 (2)	6 (4)	6 (2)	1 (1)	3 (1)	2 (2)	6 (5)
exc, d-m disagree[2]			2 (2)		1 (1)		1 (0)	1 (1)
exc, m-d disagree[2]								
d-m, m-d disagree[2]			1 (0)					
only exc[3]	5 (3)		4 (4)	3 (3)	2 (2)	1 (1)	7 (6)	7 (7)
only m-d[3]		9 (1)		7 (3)		2 (1)		3 (1)
only d-m[3]	3 (1)	7 (0)	1 (1)	3 (0)	1 (1)		1 (0)	1 (0)
exc disagrees[4]	1 (0)		2 (2)	1 (1)	1 (0)		2 (2)	
m-d disagrees[4]	2 (2)				2 (2)		3 (3)	1 (1)
d-m disagrees[4]	4 (3)	1 (1)	1 (1)			2 (2)	2 (2)	3 (3)
all disagree			1 (0)					
Total	88 (77)	88 (53)	72 (66)	72 (55)	66 (64)	66 (61)	91 (88)	91 (84)

[1] this includes cases where all three methods did not assign a parent

[2] the method not mentioned was unable to assign a parent

[3] other methods were unable to assign a parent

[4] methods not mentioned agreed on a result

Table 4-3. Genetic variation present at each locus. The mean number of alleles, and overall exclusion probabilities, are given in the 'total' row.

Locus	Individuals	# Alleles	Paternal Exclusion	Parental Exclusion
AGLA232	296	6	0.434	0.255
AGLA269	289	4	0.427	0.263
BBJ24	296	3	0.352	0.208
BM2830	296	6	0.339	0.190
BM1225	296	5	0.305	0.143
BM1824	295	6	0.341	0.181
BM3507	295	3	0.323	0.189
BM4440	296	3	0.282	0.167
BM5004	296	4	0.305	0.143
BM723	296	3	0.330	0.191
BM757	295	3	0.317	0.179
BMC1222	295	3	0.308	0.178
BOVFSH	296	9	0.472	0.286
CSSM022	296	5	0.466	0.295
Eth152	295	4	0.469	0.298
NVHRT30	292	4	0.448	0.275
RT29	296	7	0.530	0.351
TGLA126	296	7	0.509	0.333
TGLA261	296	6	0.507	0.330
TGLA53	292	4	0.348	0.187
TGLA57	296	6	0.462	0.293
Overall		4.81	0.99998	0.9967

Table 4-4. Number and proportion (in brackets) of maternity and paternity assignments over the four-year study.

	Maternity Assigned	Paternity Assigned	Total
1996	77 (0.875)	53 (0.602)	88
1997	66 (0.917)	55 (0.764)	72
1998	64 (0.970)	61 (0.924)	66
1999	88 (0.967)	84 (0.923)	91
Total	295 (0.931)	253 (0.798)	317

Table 4-5. Number of reproductively successful animals over the study period. Numbers in brackets are proportions of the total.

Table 4-5A. Males.

	1996	1997	1998	1999	Total
Paternities assigned	53	55	61	84	253
Successful males	26 (0.36)	27 (0.35)	36 (0.41)	40 (0.40)	62 (0.61)
Mature males	72	78	87	100	101
Calves/successful male	2.04	2.04	1.67	2.10	4.08
Variance	1.88	1.42	1.65	3.43	14.01
Maximum calves	7	4	7	11	24

Table 4-5B. Females.

	1996	1997	1998	1999	Total
Successful females	77 (0.66)	66 (0.54)	64 (0.50)	88 (0.70)	121 (0.81)
Mature females	116	122	129	126	150

Table 4-6. Mean proportion of calves produced per year by males in each age class. Age is taken at the time the calf was conceived.

Age Class	Mean	Variance
4-	0	-
5	0.00113	0.0000134
6	0.00300	0.0000461
7	0.0104	0.000339
8	0.0190	0.000360
9	0.0174	0.000743
10	0.0164	0.00125
11	0.0167	0.000809
12	0.0189	0.000488
13	0.00758	0.000115
14	0.0186	0.0000233
15+	0	-

Table 4-7. Mean number of calves produced per year by females in each age class. Age is taken at the time the calf was conceived.

Age Class	Mean	Variance
1	0	-
2	0.048	0.046
3	0.628	0.239
4	0.462	0.255
5	0.794	0.168
6	0.667	0.232
7	0.760	0.190
8	0.800	0.166
9	0.786	0.175
10	0.750	0.194
11	0.826	0.150
12	0833	0.147
13	0.625	0.250
14	0.786	0.181
15	0.636	0.255
16	0.727	0.218
17	0.500	0.286
18	0.429	0.286
19	0.167	0.167
20	0.333	0.333
21+	0	-

Table 4-8. The age at first reproduction for males and females who matured at the start of the study. Age is taken in the year the calf was conceived. Animals listed in the '-' column did not reproduce.

Table 4-8A. Males.

Year of Birth	Age at first reproduction				
	5	6	7	8	-
1990		1	3	3	2

Table 4-8B. Females.

Year of Birth	Age at first reproduction				
	2	3	4	5	-
1993	1	5		2	1
1994		2	3	1	

Table 4-9. Average pairwise relatedness (*R*) values for reproductive and non-reproductive pairs in each of the study years.

		R	Variance
1996	Reproductive	-0.0090	0.0196
	Non-reproductive	-0.0101	0.0277
1997	Reproductive	0.0247	0.0206
	Non-reproductive	-0.0101	0.0259
1998	Reproductive	0.0102	0.0237
	Non-reproductive	0.0066	0.0270
1999	Reproductive	-0.0027	0.0270
	Non-reproductive	-0.0077	0.0267

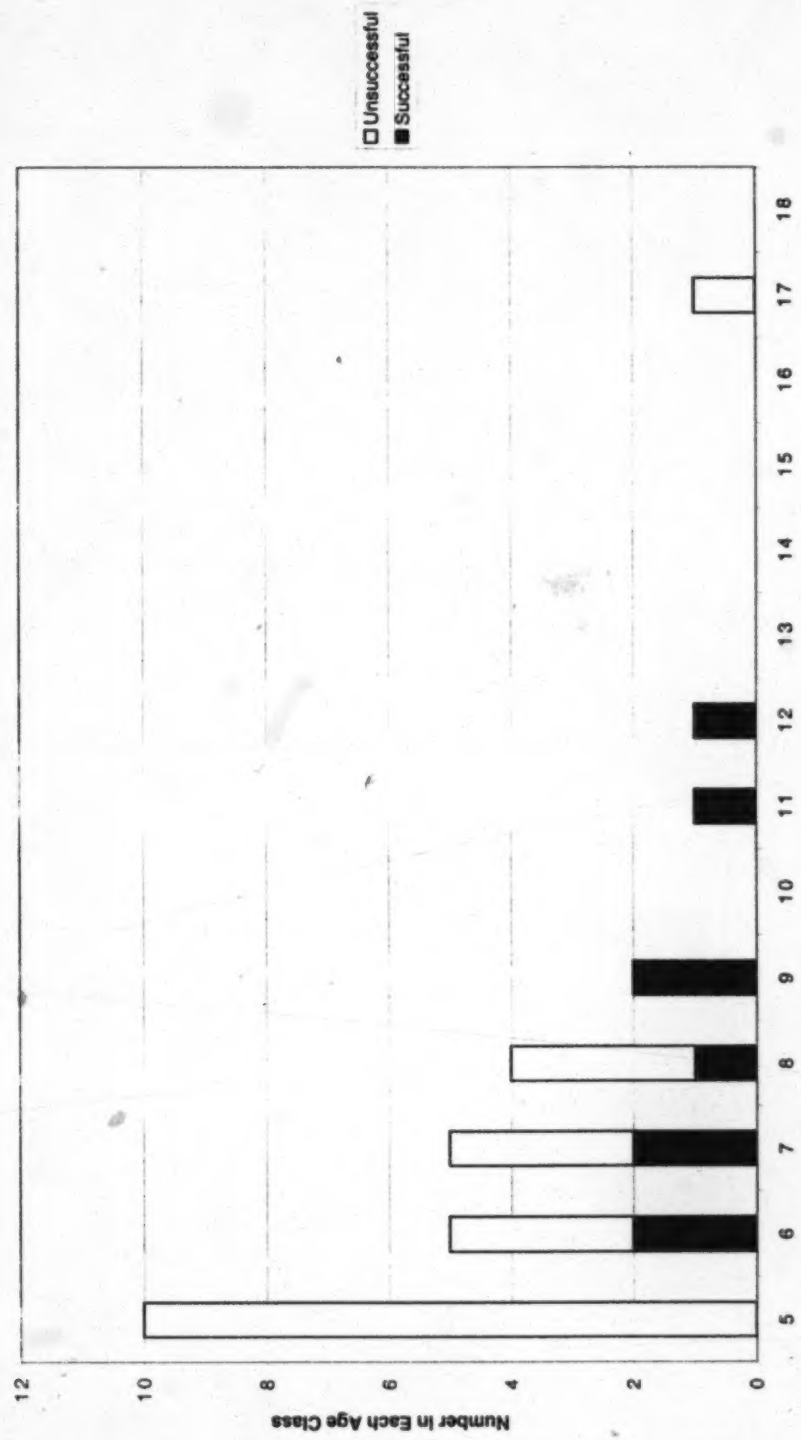

Figure 4-1A. The age structure of reproductively successful and unsuccessful males in 1996.

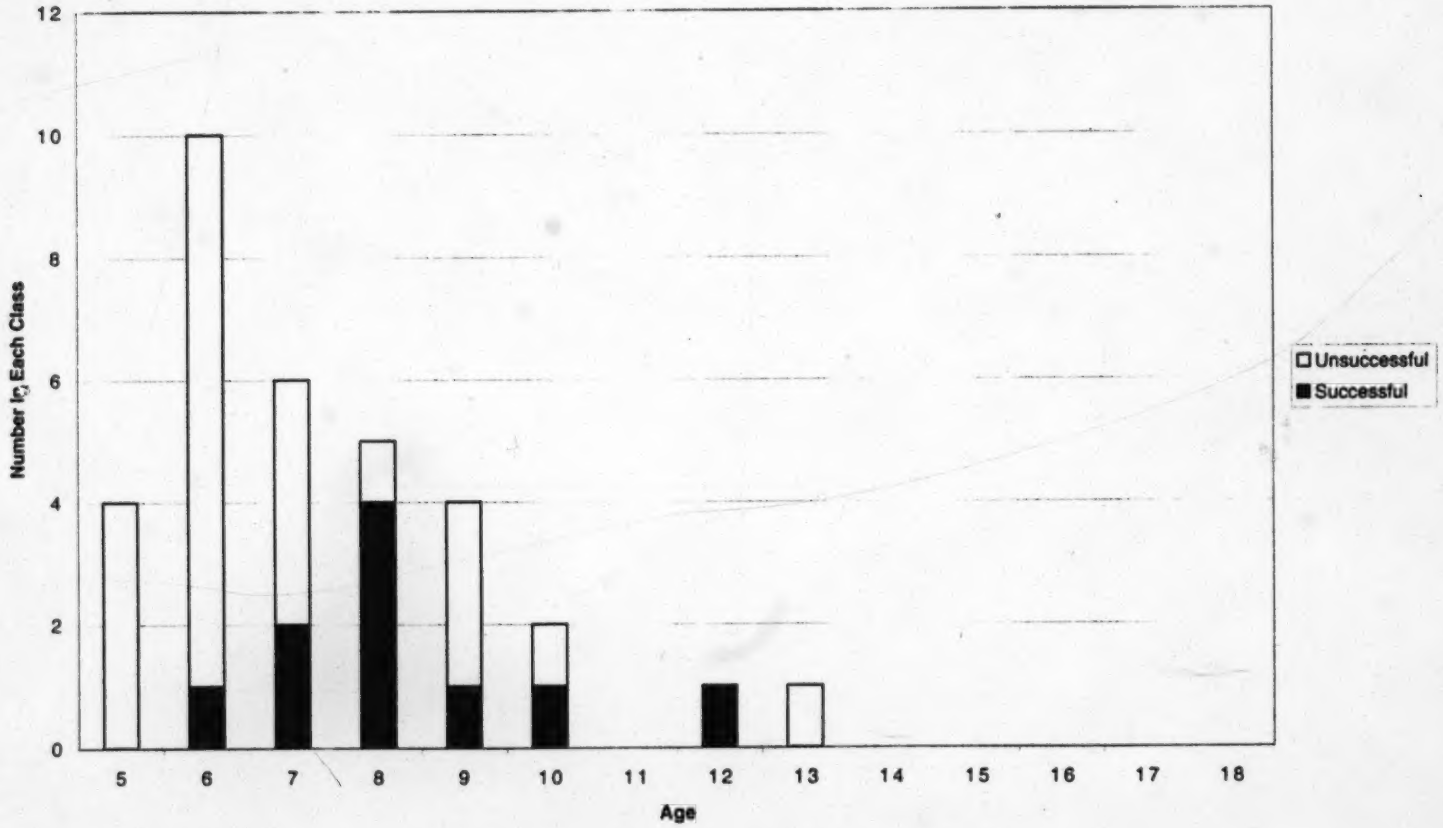

Figure 4-1B. The age structure of reproductively successful and unsuccessful males in 1997.

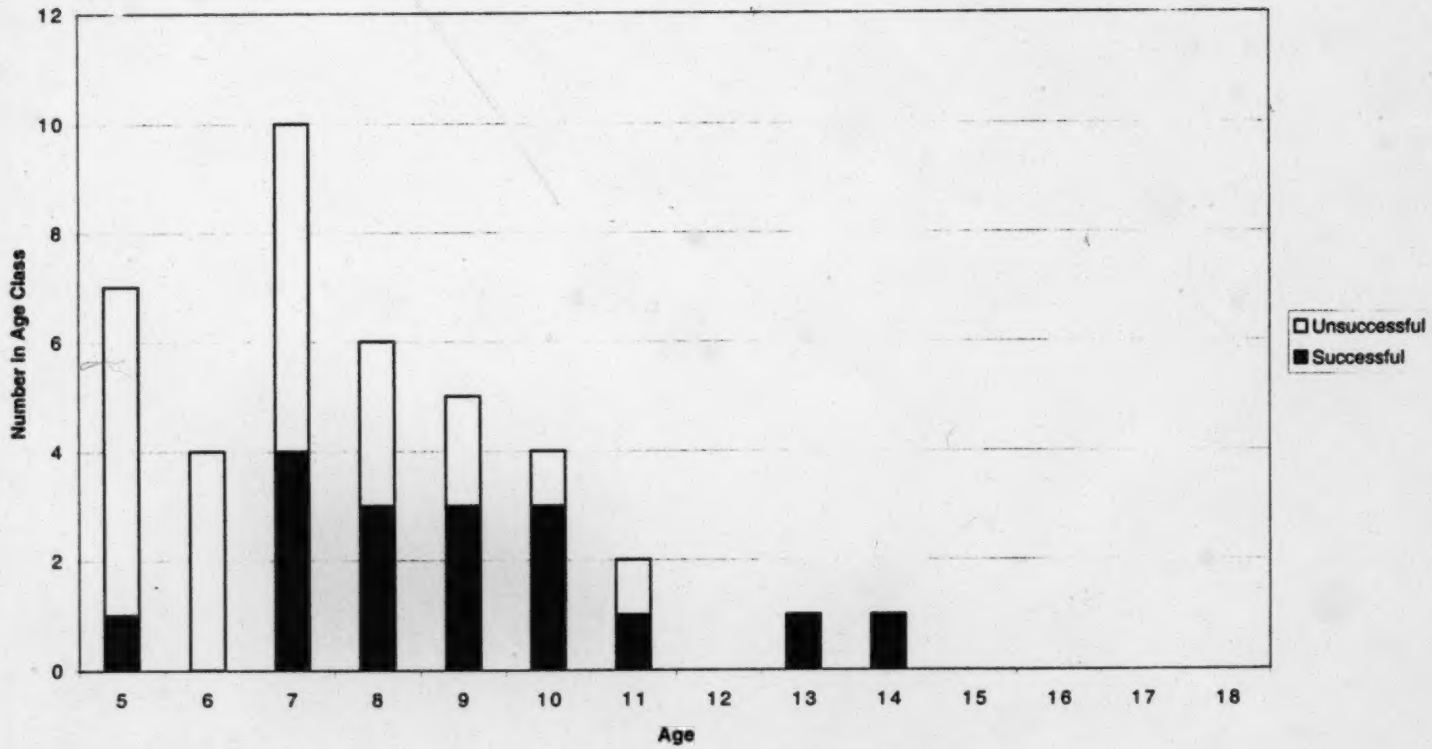

Figure 4-1C. The age structure of reproductively successful and unsuccessful males in 1998.

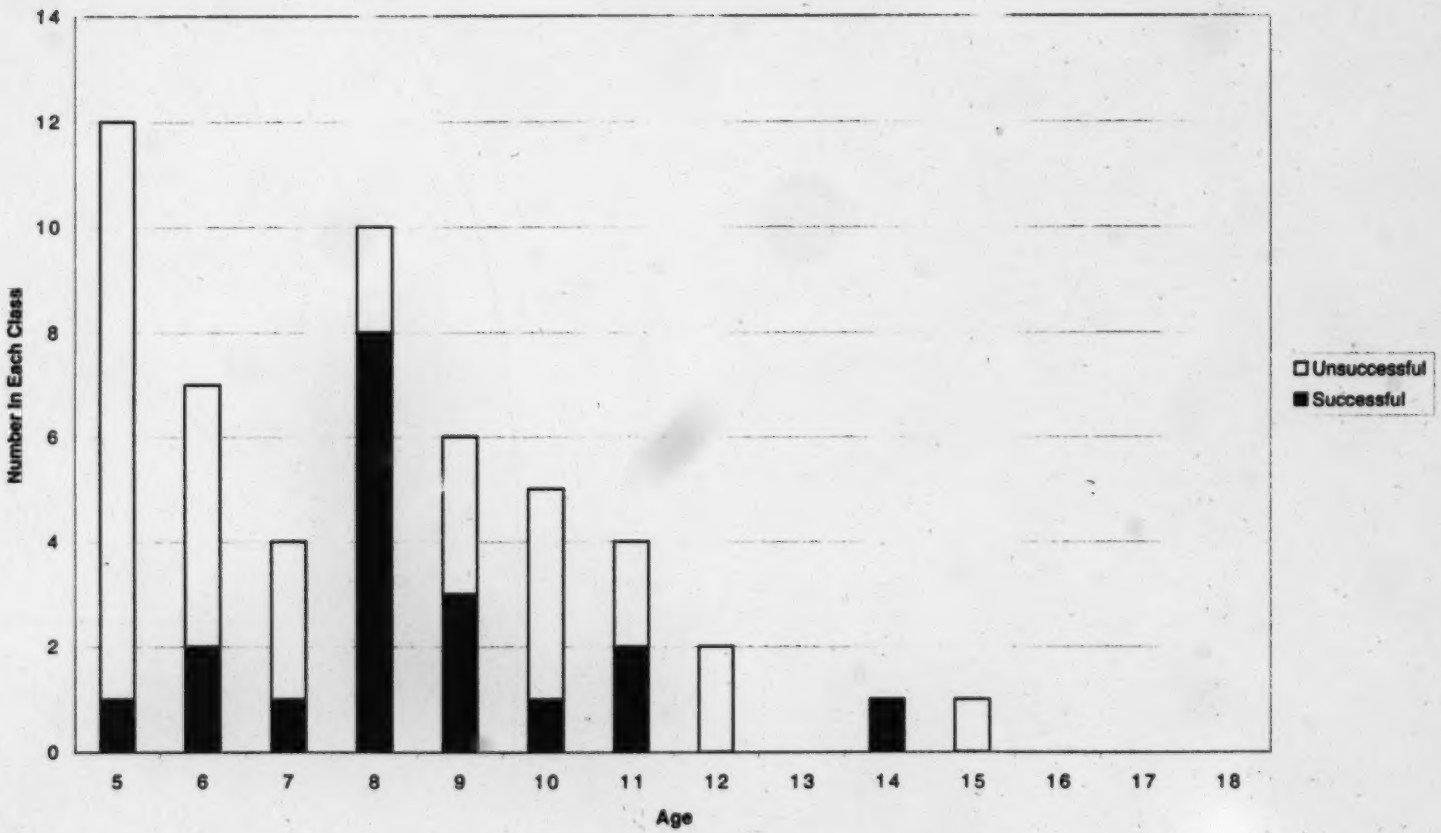

Figure 4-1D. The age structure of reproductively successful and unsuccessful males in 1999.

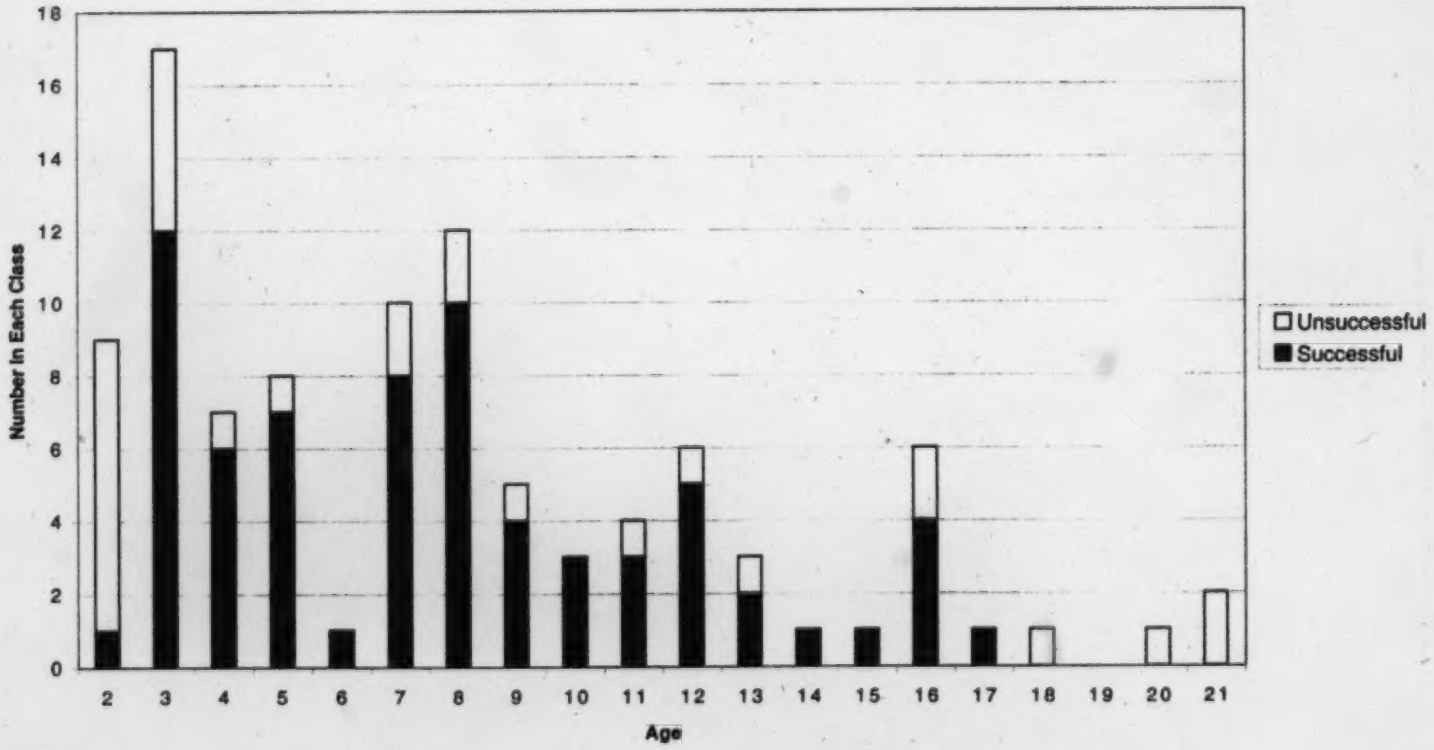

Figure 4-2A. The age structure of reproductively successful and unsuccessful females in 1996.

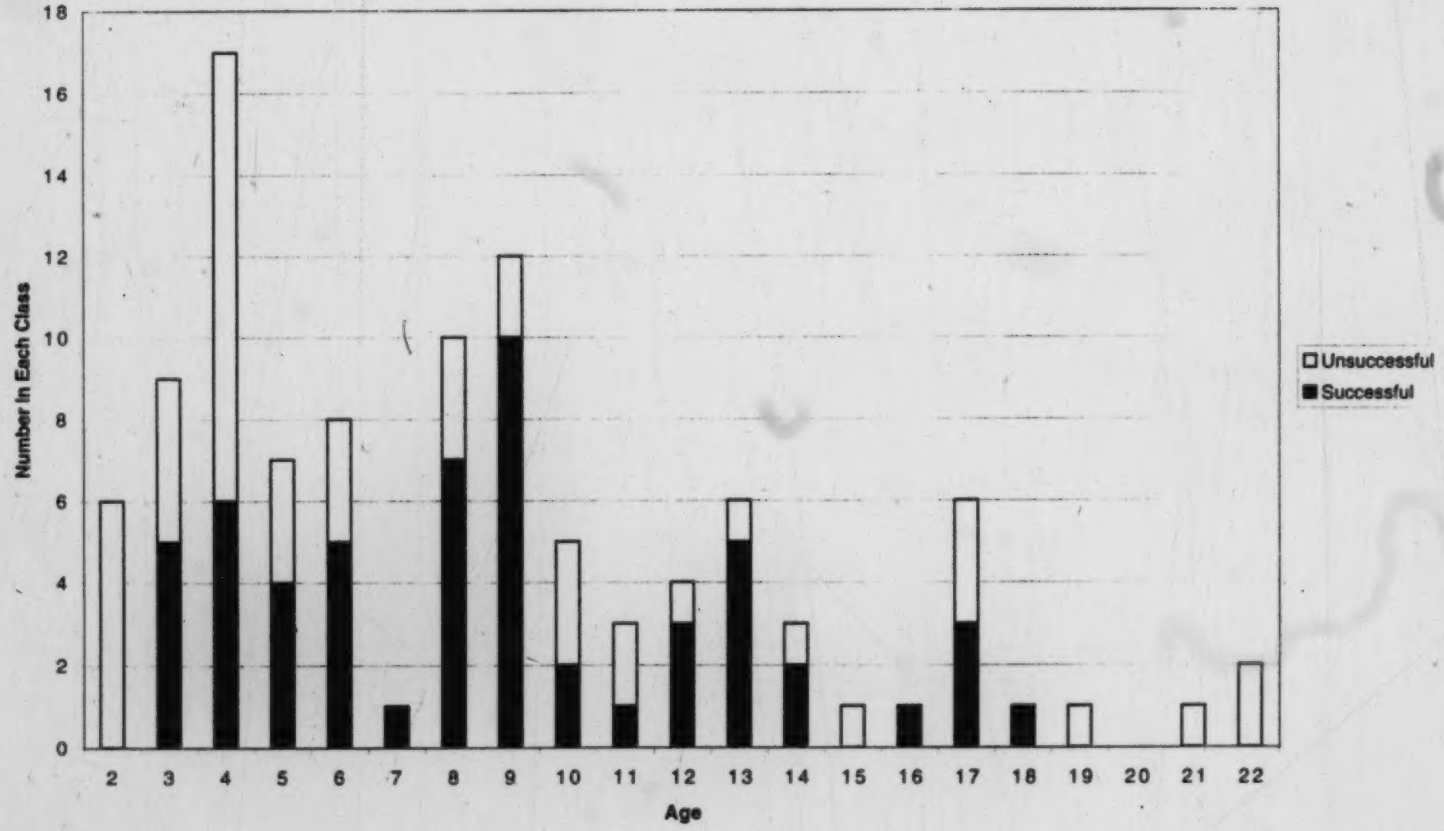

Figure 4-2B. The age structure of reproductively successful and unsuccessful females in 1997.

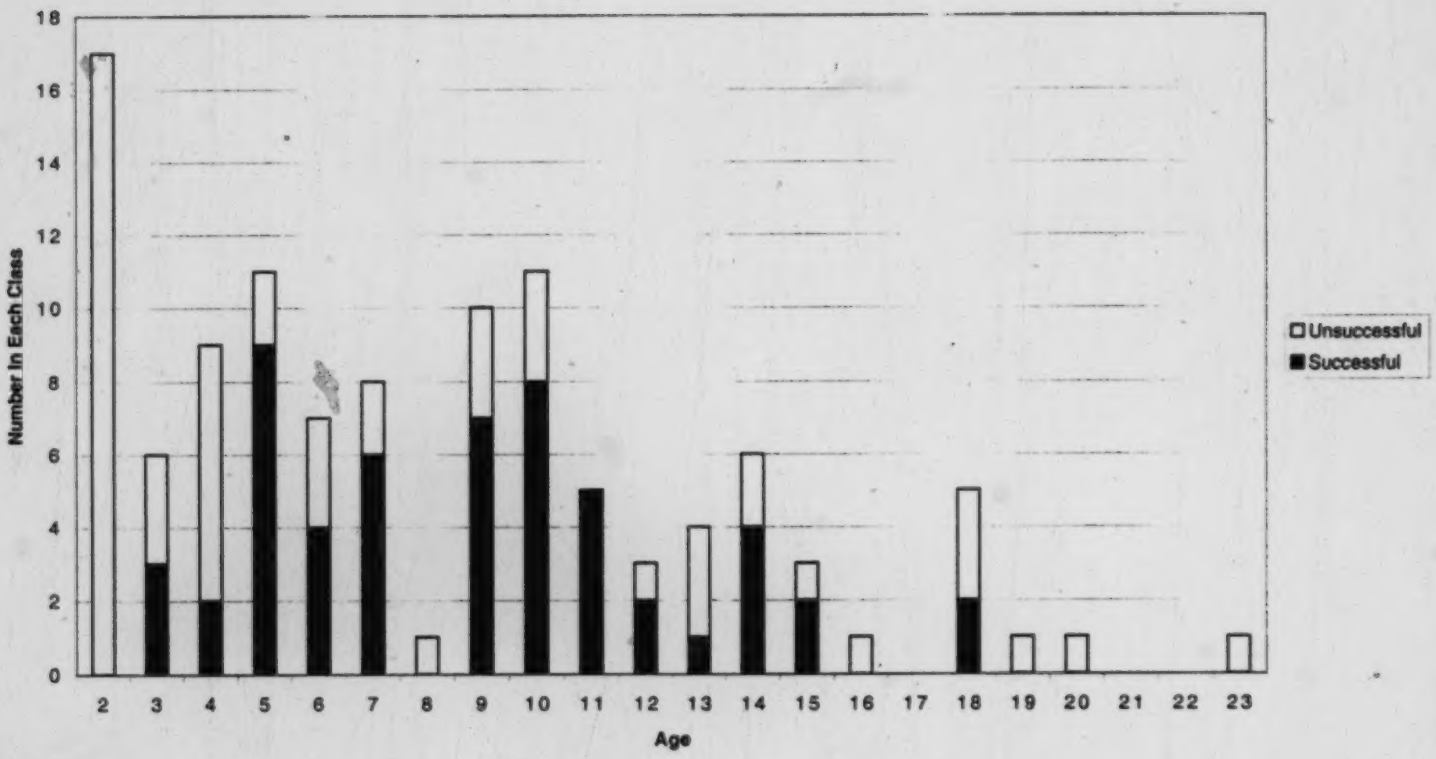

Figure 4-2C. The age structure of reproductively successful and unsuccessful females in 1998.

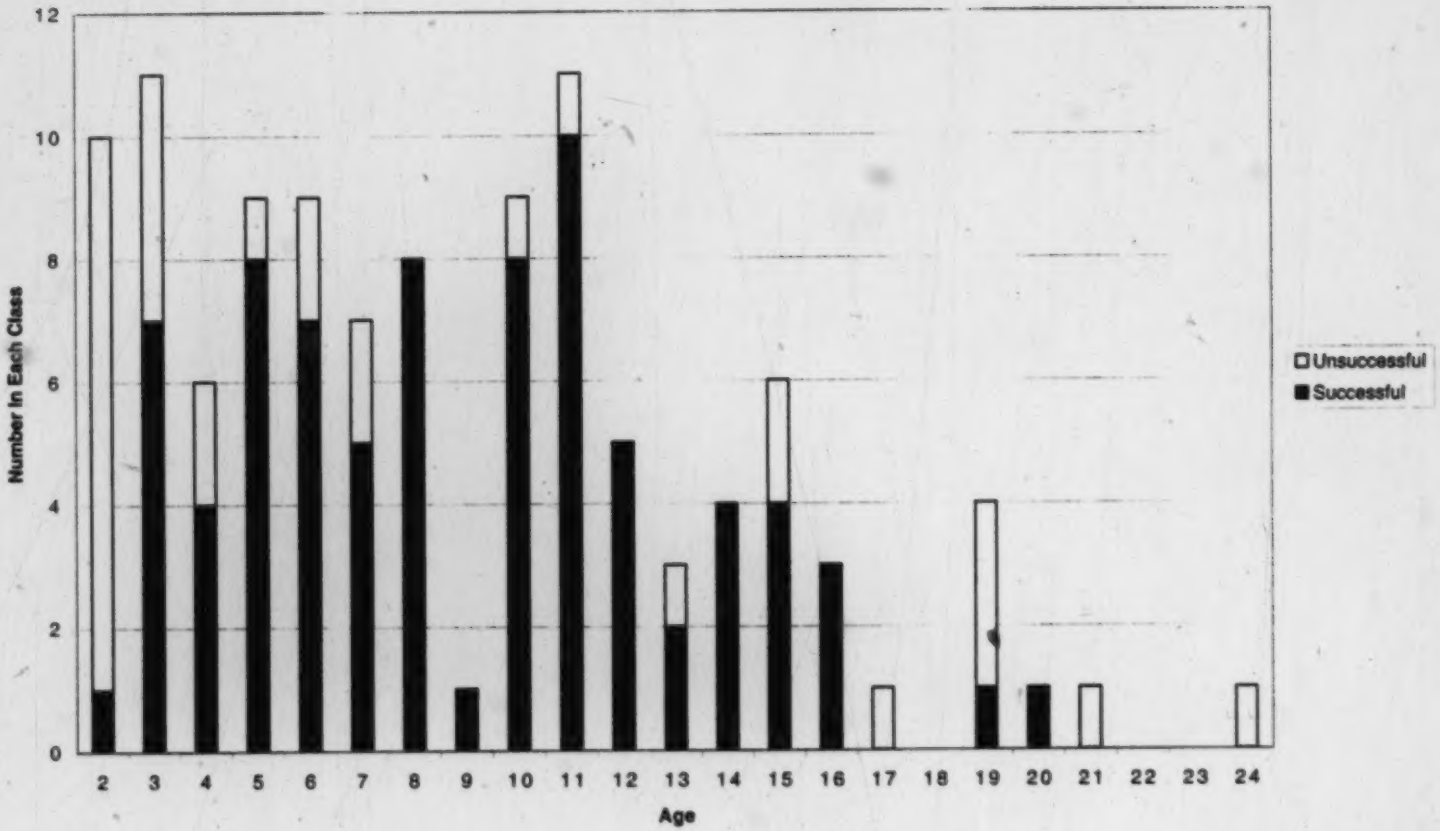

Figure 4-2D. The age structure of reproductively successful and unsuccessful females in 1999.

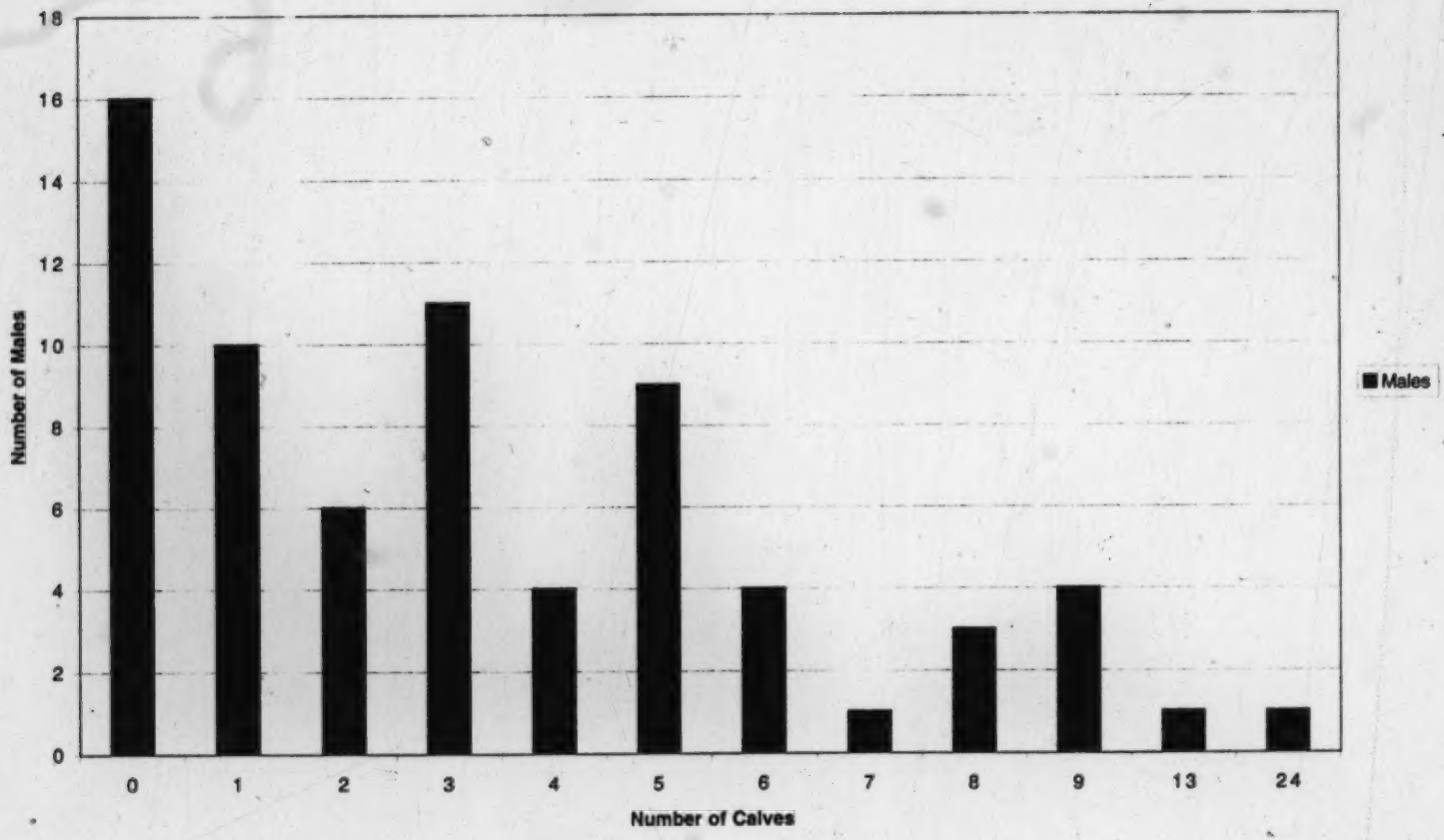

Figure 4-3A. Number of calves produced per male over the four year study period.

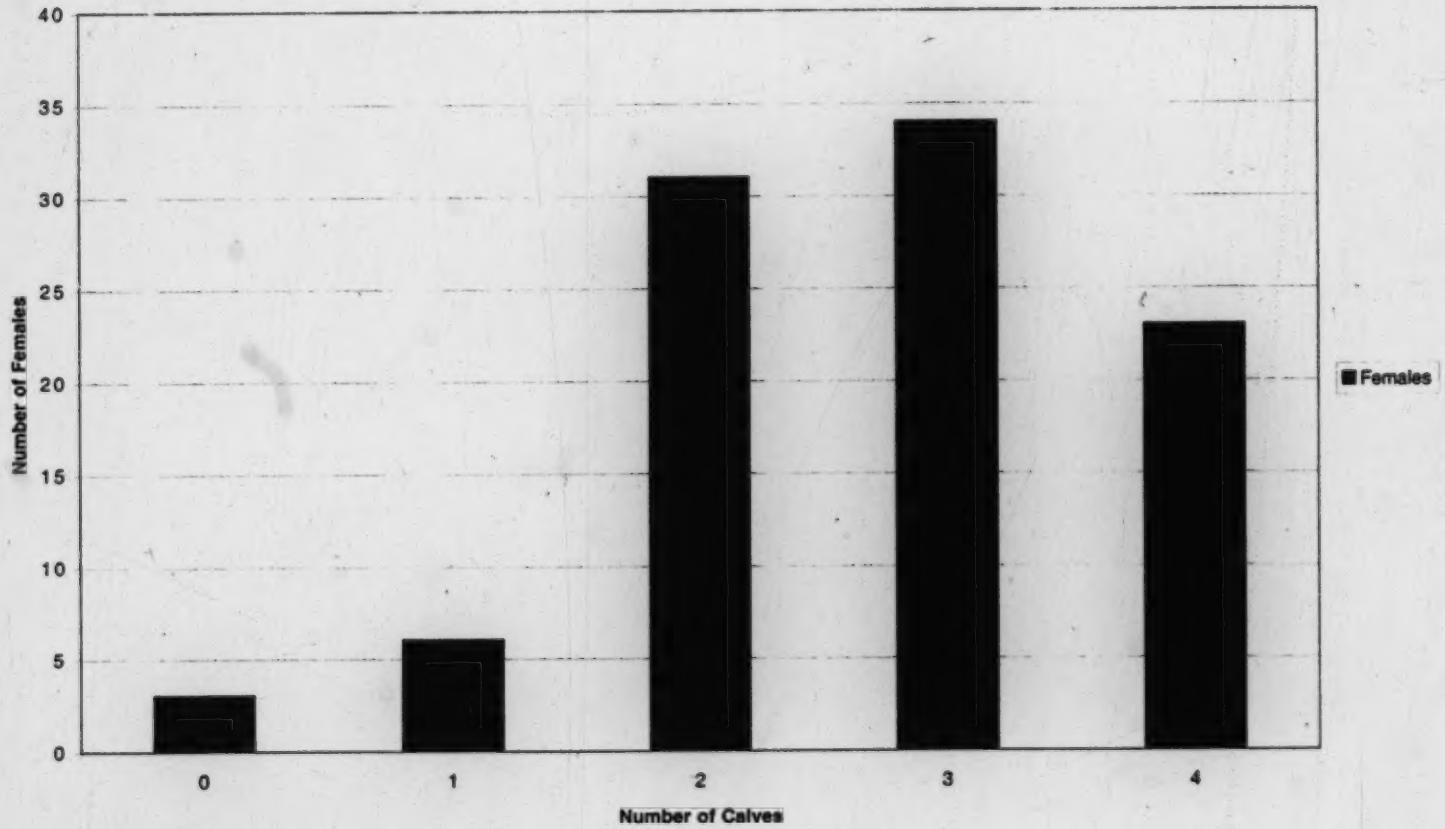

Figure 4-3B. Number of calves produced per female over the four year study period.

Chapter 5

Synthesis

The field of conservation has been greatly benefited by the use of genetic techniques to examine various conservation issues. However, the ability of genetics to aid conservation studies is affected by the genetic system used, and its ability to detect variation within and between different populations, subspecies, or species. There are a few commonly examined major themes in conservation genetics research. One such theme is the use of genetic systems to examine the phylogeny of different groups. Knowing the relationships between different taxonomic units can aid in determining which groups are most in danger of becoming extinct by placing populations into taxonomic units, and a higher conservation priority can be assigned to groups that are more distinct (see for e.g. Karl and Bowen 1999). Conservation genetics can also be used to advise in the proper management of wild populations. The amount of gene flow, and the relationships between populations, can be determined so that management decisions do not alter the natural processes in these populations. Finally, conservation genetics can be used when establishing management strategies for captive populations. These populations can be monitored for loss of genetic variation due to population bottlenecks or genetic drift. Studies of parentage, and the apparent causes of differences in reproductive success between individuals in these populations, can aid in deriving captive breeding management strategies.

This thesis used microsatellites, hypervariable nuclear DNA markers, as a tool to examine a number of conservation genetic issues related to these major themes in bison. Due to their high levels of variation, microsatellites are generally not the best method for

examining deep taxonomic splits (see discussion below). However, they can still be used to draw some conclusions about the relationships between different taxonomic units. The relationship between wood and plains bison based on microsatellite analysis was examined in an attempt to determine the type of conservation unit that best describes these subspecies. At the wild population level, data derived from microsatellite analysis was also used to elucidate what effect the various bottlenecks and animal exchanges between bison populations has had on the levels of genetic variation and relatedness among these populations. Conservation genetic questions addressing captive bison populations were also examined with microsatellite data. The relationship between genetic variation in a source population, variation in a founded population, and the amount of founders was compared in a number of bison populations to determine the ideal number of founders that would obtain a large amount of the genetic variation present in a source bison population. A recently founded population like the Hook Lake Wood Bison Recovery Project is in danger of quickly losing genetic variation via differential reproductive success. Knowledge of the breeding success of bison in captive populations, and the determinants of this success, would enable breeding strategies to be developed that would limit the loss of genetic variation in these populations.

Microsatellites in Bison

Prior to this study, microsatellites had been successfully used to answer many conservation genetics questions like those outlined above, as evidenced by the diversity of articles found in the journal *Molecular Ecology*. The isolation of some microsatellites in bison is outlined in Chapter 6. The usefulness of microsatellites to detect genetic variation was also evident in my study. In Chapters 2 and 3, I describe how these highly

variable markers allowed me to determine the genetic distinctness of a number of wood and plains bison populations. Prior studies using nuclear genetic markers in bison revealed little variation, and no differentiation among populations (see Chapter 1). In contrast, microsatellites were sensitive enough to detect subtle differences in the recent history of populations (see Chapter 2). Three of the populations examined in this Chapter were founded fairly recently from other studied populations. About 30 years ago, the wood bison populations at Elk Island National Park and Mackenzie Bison Sanctuary were founded from Wood Buffalo National Park, and the Pink Mountain population was founded from the Elk Island National Park plains bison population. Assignment test (Paetkau et al. 1995) and genetic distance (Nei 1972, Nei 1973) calculations performed on the microsatellite data were able to show that the source-founder populations were more closely related to one another than any of the other bison populations.

The ability to examine a number of variable loci inherited in a Mendelian fashion also allowed parentage to be determined in Chapters 3 and 4. As expected, the ability to determine parentage increased with the number of loci examined. For example, the Hook Lake Wood Bison Recovery Project, examined in Chapter 3, had moderate levels of genetic variability, at least compared to other bison populations. In this herd, maternity was usually known and there were only a small number of possible fathers. Therefore, only 11 loci were required to establish paternity in most cases. In contrast, the Elk Island National Park population examined in Chapter 4 had low levels of variation, unknown maternity, and a large number of potential fathers. The 11 loci used in Chapter 3 were unable to establish parentage for most of these calves. Instead, 21 variable microsatellite loci needed to be examined before parentage could be determined in most cases. A lack of resolving power has been noted in other studies of parentage using microsatellites (e.g., Coltman et al. 1998).

While microsatellites were able to examine a number of issues, their use should not be universal (Sunnucks 2000). Their mutation rate is so high (Weber and Wong 1993, Goldstein et al. 1995) that homoplasy (alleles that are identical in state but not identical by descent) can be extensive (Jarne and Lagoda 1996). In fact, alleles of the same length in different subspecies are often homoplasic, and not identical by descent (Estoup et al. 1995). As a result, populations separated by more than about 10 000 years seem equally related, regardless of the actual length of separation between them (Paetkau et al. 1997). For this reason, they are not good systematic tools, and cannot be relied upon for phylogenetic inferences beyond a few thousand generations (Zhivotovski and Feldman 1995, Jarne and Lagoda 1996). However, the microsatellite data can still be examined to determine if it is able to place wood and plains bison into specific conservation units.

Is *Bison bison athabascae*, An Evolutionarily Significant Unit?

Recently, some management decisions have been based on the concepts of evolutionarily significant units (ESUs) and management units (MUs). The concept of ESUs was first suggested in 1986 as a result of frustration in the inability of current mammalian taxonomy to determine which groups of organisms should be protected (Ryder 1986). Moritz (1994) has suggested that ESUs be defined genetically as groups whose mtDNA lineages are reciprocally monophyletic. It has previously been shown that this is not the case for wood and plains bison (Polziehn et al. 1996), and therefore they do not fit this definition of an ESU. However, new groups of mammals with long generation times that were founded from a parent population of even moderate size would not be expected to become monophyletic for thousands of years (Paetkau 1999). Using this genetic

definition, some separate species such as polar bears (*Ursus maritimus*) and brown bears (*Ursus arctos*) would not be granted ESU status.

MUs are defined as populations that have significant differences in allele frequencies (Moritz 1994). As discussed in Chapters 2 and 3, wood bison populations do have significantly different allele frequencies than plains bison populations. However, all bison populations are significantly different from one another. Therefore, every bison population examined in this study is equally deserving of MU status if this definition is used.

Another proposed conservation unit is the geminate evolutionary unit (GEU, Bowen 1998). Populations may be considered GEUs if they are morphologically, behaviorally, or ecologically distinct, but genetically homogenous (Bowen 1998). The GEU concept is then similar to the definition of an ESU as an "important component in the evolutionary legacy of the species" put forth by Waples (1991). The GEU is designed to protect populations which may represent new evolutionary pathways. This is a term that may apply to wood bison, as they have been described as morphologically different from plains bison (Van Zyll de Jong et al. 1995), and may have some behavioral differences during the rut (Melton et al. 1989, Chapter 4 this thesis). However, the GEU concept as currently defined is a bit nebulous to base management decisions upon, as it is impossible to predict which populations would undergo speciation over time.

The conservation unit concept, if any, which best applies to wood bison is unclear. This is in no small part due to the inability of current genetic techniques to definitively place populations within these units. I am confident that in a few years conservation unit definitions and genetic techniques will have reached a point where the identification of natural groups within species will be possible. Until such time, I suggest that wood bison be managed separately from plains bison, and conservation efforts such as that outlined in Chapter 3 be continued. Wood bison conservation has made great

headway in the last few years, and the continuation of these efforts should succeed in removing the threatened status of this group.

Application of Microsatellite Results to the Conservation Genetics of Wild Bison Populations

The use of variable genetic markers allowed the amount of diversity in wood and plains bison populations to be quantified (Chapter 2). Low levels of genetic variation have been linked to low fertility, congenital abnormalities, and a weakening of the immune response (for review see O'Brien 1994). However, it should be noted that not all populations or species suffering from low diversity seem to be negatively affected by it. For example, black bears (*Ursus americanus*) in Terra Nova National Park are the least variable of the populations surveyed by Paetkau and Strobeck (1994), but are known for their large litters and large body sizes (Payne 1978, Rich 1986). When compared to other North American ungulates for which data was available, genetic diversity in bison was higher than in moose (Broders et al. 1999), similar to wapiti (Polziehn et al. 2000), and lower than caribou (Zittlau et al. 2000). The amount of genetic diversity seen in bison is a bit surprising, given the large bottleneck undergone by this species. The collection of bison opportunistically by ranchers in the late 1800s may have allowed for the sampling of a large amount of the diversity present in bison herds at that time.

One population of bison, that at Antelope Island State Park, was identified as containing lower levels of variation than any other. Consequently, managers may wish to add animals to this population to increase its genetic diversity, especially if inbreeding effects are present. It should be noted that there are also good reasons not to endorse the introduction of bison into established populations. Once the animals have interbred, the

mixing of genes between these groups of animals is irreversible. Two examples of the exchange of bison between herds that, in hindsight, resulted in undesirable hybridization events are: the addition of plains bison to Wood Buffalo National Park in the 1920s, and the movement of bison now known to have interbred with domestic cattle from Custer State Park to a number of other populations (Polziehn et al. 1995, Ward et al. 1999, 2001).

It has been proposed that the bison indigenous to Yellowstone National Park were actually a type of bison called mountain bison, referred to as *Bison bison athabascae* (Meagher 1973). However, this taxonomic issue is in doubt (for review, see Roe 1970). If mountain bison did exist in this park, the current population should be genetically distinct from other bison populations which do not contain any mountain bison input in their gene pool, or more similar to wood bison as mountain bison and wood bison share the same subspecific designation. The genetic relationships between Yellowstone National Park and the other bison populations show that this population is more similar to plains bison than wood bison, and is therefore not deserving of increased conservation effort. The genetic relationships also showed that, as expected, populations that have been recently founded were closely related to their source population.

Application of Microsatellite Results to the Conservation Genetics of Captive Bison Populations

The sampling of a representative amount of the genetic variation present in a source population should be the conservation genetic goal during the establishment of a captive population. Four of the populations examined in Chapters 2 and 3 were founded from other of the sampled populations. About 30 years ago, the Pink Mountain population was

established from the Elk Island National Park plains bison population with 48 animals. Around this time, the wood bison populations from Elk Island National Park and Mackenzie Bison Sanctuary were established from 11and 16 animals, respectively, from Wood Buffalo National Park (Polziehn 1993). Also, the Hook Lake Wood Bison Recovery Project was founded from 58 individuals from the wild Hook Lake population between 1996 and 1998. The expected level of variation in the founded population was calculated with formula [1]

[1] $$H_1 = (1 - 1/2N_e)\, H_0$$

Effective size of the Hook Lake Wood Bison Recovery Project founder population was calculated using formula [2]

[2] $$N_e = 4\,(N_f \times N_m) / (N_f + N_m)$$

where N_m and N_f are the number of males and females, respectively. Other populations were assumed to have been founded from an equal number of males and females. Observed and predicted heterozygosities in the source and founder populations can be found in Table 5-1 and Figure 5-1. In general, populations established from a larger number of individuals contained more of the genetic variation present in the source population. Also, older founded populations contain less variation than the predicted value, due to the amount of time that genetic drift has been acting on the population. The exception to both of these trends is Elk Island National Park, which has higher levels of heterozygosity than expected, given the amount of time genetic drift has acted upon this population. It should be noted that the Elk Island National Park population appears much less variable than Wood Buffalo National Park when other measures of variability

besides heterozygosity are used. This illustrates the fact that natural populations do not always give the same results that theoretical populations do.

Another example of the benefits of being able to detect differences in genetic diversity can be found in Chapter 3. Therein, diversity in the Hook Lake Wood Bison Recovery Project was compared to that found in all other wood bison populations. Microsatellites allowed me to determine that this newly founded population was more variable than other recently established wood bison populations but did not contain a representative amount of the diversity present in Wood Buffalo National Park. Future salvage attempts can now easily be compared to Wood Buffalo National Park and the populations derived from it to determine whether the diversity in Wood Buffalo National Park has been properly represented. Without the sensitivity of microsatellites to genetic variation, this analysis would not have been possible.

A Genetic Look at Bison Mating Systems

The ability of an individual to produce offspring is a key basis of selection. While individual survival, differential reproductive output, progeny viability, and progeny quality all play a role in genetic success, I was only able to closely examine differences in reproductive output over my four year study period. One aspect of progeny viability was also considered, as calves had to survive until the annual roundup (roughly eight months after birth) to allow their parents to be considered reproductively successful that year. However, to get a true picture of progeny viability, their survivorship should be examined through adulthood.

As reviewed in Chapter 4, bison have a system of reproductive behaviour wherein dominance is temporary, and difficult to maintain. This is supported by the genetic

evidence that a large number of males are able to successfully reproduce each year, and the difficulty in predicting male reproductive success. Similar systems have been detected by mating observations in other ungulates (see for e.g. Lent 1965, Geist 1971). Such breeding systems are favoured over others such as territoriality or a stable ranking dominance when: i) habitat seasonality is high, allowing males to store energy reserves, ii) population sizes are large, making individual recognition of dominant males difficult, iii) the location of female groups is unpredictable so that a male must be in a female group to potentially obtain matings, and iv) several females are in estrous simultaneously, so that protecting all females in estrous is difficult (for review see Owen-Smith 1977). All of these conditions occur in the bison at Elk Island National Park and most, if not all, bison populations greater than a few hundred individuals. This mating system tends to have a greater physical cost than others due to high levels of competition, which could explain the fact that older males do not seem to be as successful as younger ones.

Extensive mate competition could also explain the amount of sexual dimorphism seen in this species. The idea that sex differences in size could be explained by sexual selection acting through male-male competition can be traced to Darwin (1871). Sexual dimorphism is correlated with the levels of mate competition in a number of species (Clutton-Brock et al. 1977, Jehl and Murray 1986). If larger bison males are more able to obtain and defend mates, larger size should be selected for. Weight was correlated with male reproductive success in Elk Island National Park wood bison males, suggesting that the sexual dimorphism in this species could be explained by the increased reproductive success of larger animals. However, a large amount of the variation in reproductive success could not be explained by male size, so male reproductive success must also be affected by a number of other, as yet unidentified, factors. One possible area of study would be to examine the relationship between forage quality and quantity during an individual's subadult years with reproductive success. In cattle, weight at adulthood is

affected by the amount of forage obtained during subadulthood (for review, see Bagley 1993). The variance in adult weight due to environmental factors may be masking the genetic advantages an individual has in obtaining reproductive success.

Future Studies

Microsatellites are highly variable, and are therefore a useful tool for determining the amount of variation present in a population. However, as they are thought to be non-coding, the examination of diversity with microsatellites may not be the best method for examining the genetic adaptability of a population. An alternative would be to evaluate loci known to code for a biological function. For example, the major histocompatibility complex's (MHC) function is to allow the immune system to identify and destroy virus-infected cells (Klein 1986). Measuring the diversity at the MHC loci may give a better indication of a population's ability to stave off infection, and could be examined in populations such as Antelope Island State Park where microsatellites have revealed low levels of genetic variation. It should be noted, however, that variation at the MHC is likely selected for, so it is not a good indicator of diversity throughout the rest of the genome.

In Chapter 3, I noted that the genetic variation present in the Hook Lake Wood Bison Recovery Project, Elk Island National Park, and the Mackenzie Bison Sanctuary populations is less than that found in the Wood Buffalo National Park population. This has important implications for the conservation of wood bison in Canada, in that the diversity present in the Wood Buffalo National Park population is not completely represented in the other populations. Therefore, future salvage operations should be performed in an attempt to sample the diversity present in Wood Buffalo National Park.

The genetic techniques outlined in Chapter 3 should be used to examine genetic variation in any future salvage operation, and estimate the amount of the diversity in Wood Buffalo National Park represented in its daughter populations. Genetic monitoring of recovery populations over time is also a key issue for continuing research. Genetic drift could result in the loss of variation in recovery populations, especially if there is differential reproductive success. Therefore, these populations should be examined at various times to determine how much diversity they maintain over time.

By far the most potential for future studies stems from Chapter 4. This section examines the relationship between reproductive success and a number of other factors, such as success in prior years, weight, and age for males and females. However, many more questions can be investigated if this study were to continue. This thesis only looked at a four year snapshot of reproductive success. Sampling of calves over the next ten or 20 years would allow lifetime reproductive success, and each cohort's peak reproductive years, to be determined. An investigation of inbreeding in this population is another issue that could be examined. Next year, most of the female calves born at the beginning of this study will breed. Paternity of their calves can be established to determine if females are more or less likely to breed with their fathers than expected by chance. As mentioned above, one aspect of individual fitness is the quality of progeny. If the investigation of parentage at Elk Island National Park is continued over a generation, then the progeny quality can be determined by examining the number of 'grandchildren' each of the original animals in the study has. In this way, the relationship between fitness across generations can be determined. As well, the comparison of more demographic and individual data with reproductive success may increase the ability to predict this success.

Microsatellites have proven to be useful tools in examining a number of ecological questions in bison. While this section illustrates that there are some issues that can still be addressed using this technology, this thesis successfully used microsatellites

to examine a number of issues pertaining to bison ecology, from the individual to the population level.

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Table 5-1. Observed heterozygosities (H_O) in founding populations and their source populations, and the predicted heterozygosity (H_P) in the founding population, given its number of founders (N_e).

Source	HLR	H_O	0.532
Founding	HLA	N_e	44.5
		H_O	0.508
		H_P	0.526
Source	WBNP	H_O	0.552
Founding	EINPW	N_e	11
		H_O	0.520
		H_P	0.527
Source	WBNP	H_O	0.552
Founding	MBS	N_e	16
		H_O	0.441
		H_P	0.535
Source	EINPP	H_O	0.560
Founding	PM	N_e	45
		H_O	0.516
		H_P	0.554

Note: population abbreviations: wild Hook Lake Region (HLR), captive Hook Lake population (HLA), Wood Buffalo National Park (WBNP), wood bison at Elk Island National Park (EINPW), Mackenzie Bison Sanctuary (MBS), plains bison at Elk Island National Park (EINPP), and Pink Mountain (PM).

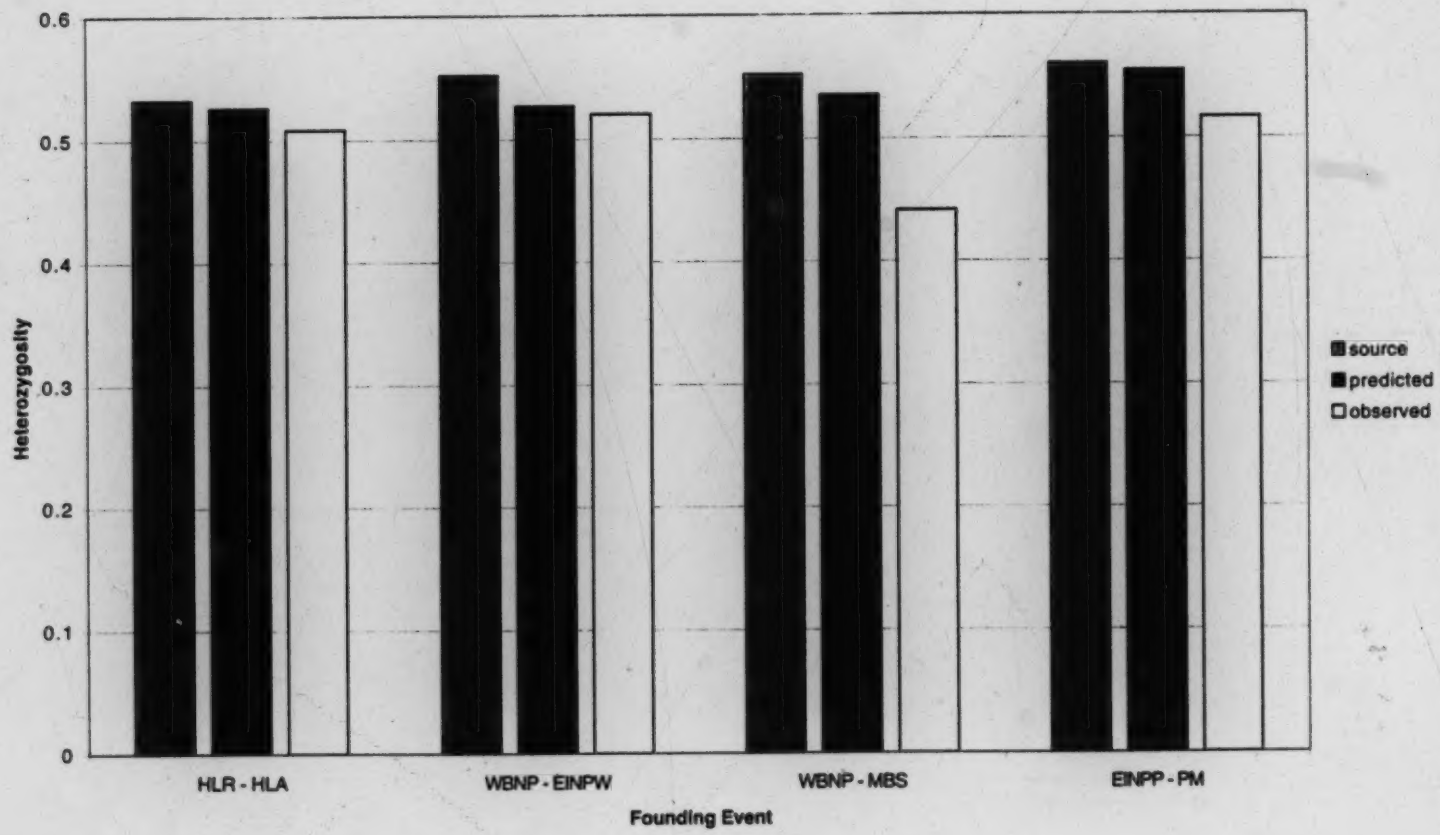

Figure 5-1. Predicted and observed heterozygosity values after a founding event.

Chapter 6

Addendum-The Isolation and Characterization of Microsatellite Loci in Bison, and their Usefulness in Other Artiodactyls[1]

Source/Description

Wood bison (*Bison bison athabascae*) are currently considered a threatened species in Canada. Previous microsatellite studies, using loci isolated from domestic cattle (*Bos taurus*) and caribou (*Rangifer tarandus*), have revealed little variation in the wood bison population at Elk Island National Park (1). A parentage study of these bison is currently ongoing by the authors, which requires a larger number of variable microsatellite loci. Some studies have suggested that microsatellite loci are more variable in the species from which they are characterized (2). Therefore, four microsatellite loci were isolated from wood bison at Elk Island National Park. Amplification of these loci in plains bison (*Bison bison bison*) from Custer State Park, one of the most variable bison populations (1), was also performed. As cross-species amplifications of microsatellite loci have proven successful in artiodactyls, amplifications were also attempted in a number of other members of this order (3, 4).

Bison genomic DNA was isolated from whole blood samples of animals from Elk Island National Park (kindly supplied by Bruce Rutley). Creation of a genomic library was performed as in Paetkau & Strobeck (5). Screening of the library was performed using a synthetic $(dT\text{-}dG)_{11}$ oligonucleotide probe and a biotin detection kit (BRL).

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[1] A version of this chapter has been published. Wilson, G.A., and Strobeck, C. 1999. Anim. Genet. **30**: 226-227.

Twenty positive clones resulted, and all were sequenced as per Zheng *et al.* (6), except sequencing was performed on an ABI 377 Automated Sequencer. Primers were designed to amplify seven of these microsatellite loci using the program OLIGO (National Biosciences, V 4.0). Four of these primer sets yielded products of the expected length in the Elk Island National Park and Custer State Park bison populations. These loci are shown in Table 1.

PCR conditions

The PCR reactions for these amplifications included 0.3 units of *Taq* polymerase (isolated as in 7), 120 μM dNTPs, 0.16 μM each primer 2 mM $MgCl_2$, PCR buffer (50 mM KCl, 10 mM Tris buffer, pH 8.8, 0.16 mg/mL BSA and 0.1% Triton X100) and approximately 60 ng of genomic DNA in a final reaction volume of 15 μL. The microsatellite loci were amplified in a Perkin Elmer 9600 thermal cycler using the following cycling conditions: 1 min at 94°C; three cycles of 30 s at 94°C, 20 s at 54°C, 5 s at 72°C; 33 cycles of 15 s at 94°C, 20 s at 54°C, 1 s at 72°C; ending with 30 s at 72°C. Fluorescently labelled products were detected after electrophoresis on an ABI 373 A Automated Sequencer. These microsatellite loci, isolated from bison, were found to be no more variable in the Elk Island National Park and Custer State Park populations than those loci isolated from domestic cattle or caribou (1).

Comments

Amplification of these loci were also attempted in two other bovid (bighorn sheep and mountain goat), and four cervid (moose, elk, caribou, and mule deer) species. All individuals tested were from the same population, or geographic area. PCR conditions were identical to those above, except the amount of *Taq* polymerase was raised to 0.35 units for all amplifications of *BBJ 3*. Amplification of some of the loci were successful in all species, however, not all species displayed variation at these loci (Table 2).

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Table 6-1. Primer sequences and other characteristics of the bison microsatellite loci. First values for number of alleles and expected heterozygosity are for the Elk Island National Park, and second values are for Custer State Park. Values in brackets are the number of individuals surveyed at that locus. Size range refers to the observed size, in base pairs, of the PCR products. Unbiased expected heterozygosity was calculated using the formula from Nei & Roychoudhury (8).

Locus	Repeat motif	Primer sequences	Size range	Total No. of alleles	no. of alleles	Expected heterozygosity	Accession number
BBJ 2	$(TG)_9CC(TG)_3$	ACA CTG CCC CGG TAT CTT TG	170	1 (35)	1	0	AF095598
		GCA CTT TAG CTC ACT TCC TG		1 (30)		0	
BBJ 3	$(GT)_{12}C(TG)_2$	TTA GCC CAA TCT CAG AGT TG	136-142	2 (35)	3	0.073	AF095599
		GGC ATG TCC TGT GGA CCA A		3 (30)		0.269	
BBJ 11	$(TG)_5GG(TG)_7$	AGG TTA ATG TTT TCC CAG TT	167-171	3 (34)	3	0.482	AF095600
		AAA TGG CAG CCC ACT CCA AT		3 (30)		0.581	
BBJ 24	$(TG)_{14}$	GAG GAT TAT GGG GAC ACT GC	254-266	3 (34)	5	0.674	AF095601
		TAC TGG TCA CAA CAC TTC AC		4 (29)		0.673	

Table 6-2. Results of the cross-species amplifications using the bison loci. Size range is in base pairs. Numbers in the brackets are the number of individuals sampled. Individuals were always from the same population. Weak amplifications are denoted by 'Δ', and multi-banding patterns are denoted by '§'.

	Locus	Moose *Alces alces*	Elk *Cervus elaphus*	Caribou *Rangifer tarandus*	Mule deer *Odocoileus hemionus*	Mountain goat *Oreamnos americanus*	Bighorn sheep *Ovis canadensis*
Size range	*BBJ 2*	168	172	176-178	176-188	168-172	176
No. of alleles		1 (5)	1 (5)	2 (5)	4 (5)	2 (5)	1 (5)
Size range	*BBJ 3*	140-148	§	138-140	§	153-155	§
No. of alleles		3 (3)Δ		2 (5)		2 (5)	
Size range	*BBJ 11*	160	158	168-180	158-160	140	142
No. of alleles		1 (5)	1 (5)	7 (5)	2 (5)	1 (5)	1 (5)
Size range	*BBJ 24*	§	§	§	275-279	§	§
No. of alleles					2 (2)Δ		